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(54) IDENTIFICATION OF A NOVEL HUMAN RECEPTOR TYROSINE KINASE GENE

IDENTIFIZIERUNG EINES MENSCHLICHEN REZEPTOR-TYROSINKINASEGENS

IDENTIFICATION D'UN NOUVEAU GENE HUMAIN RECEPTEUR DE TYROSINE KINASE

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Description**FIELD OF THE INVENTION**

5 [0001] This invention relates to the DNA sequence encoding a novel human growth factor receptor which is a type III receptor tyrosine kinase. The receptor is referred to as Kinase insert Domain containing Receptor (KDR) and binds specifically to the growth factor vascular endothelial cell growth factor (VEGF). This invention also relates to the amino acid sequence of the receptor, an expression vector comprising the DNA sequence, a lambda gt11 phage, primers, a method for the expression of the protein encoded by the DNA sequence, and the use of a biologically active human type III receptor tyrosine kinase in a screening of pharmaceuticals for antagonist or agonist VEGF action on the human type III receptor tyrosine kinase

BACKGROUND OF THE INVENTION

15 [0002] Growth factors are small molecules which regulate normal cell growth and development through interaction with cell surface receptors. The receptors for a number of growth factors are referred to as tyrosine kinases; that is, binding of growth factor to the receptor stimulates an increased phosphorylation of tyrosine amino acids within the receptor; this in turn leads to cellular activation (Bibliography 1).

20 [0003] There is increasing evidence that genetic alterations affecting the expression of receptor tyrosine kinases (RTK) can contribute to the altered cell growth associated with cancer. This conclusion is supported by the frequent identification of RTK as products of the oncogenes for many of the acutely transforming retroviruses (e.g., 2,3,4) and the overexpression of RTK in certain cancers (5). The identification of a novel RTK may lead to a better understanding of cell growth under both normal and transforming circumstances.

25 [0004] The amino acid sequence in the catalytic domain of all tyrosine kinases has been conserved (6). Detailed analysis of the amino acid sequences within the catalytic and noncatalytic domains of RTK indicates the existence of distinct structural subtypes. One group of RTK (designated type III) includes the ckit proto-oncogene and the receptors for platelet derived growth factor (PDGF) and colony stimulating factor-1 (CSF-1).

30 [0005] The most unusual feature of this subtype is that its catalytic (kinase) domain is interrupted by a long insertion sequence of 12-102 amino acids (the kinase insert domain). The two peptides constituting the kinase domain are conserved between the receptors, while the sequence of the kinase insert domain is unique for each receptor.

35 [0006] Several approaches have been tried in order to identify novel RTK, including low-stringency screening of cDNA libraries with previously characterized DNA probes (7). More recently, a technique has been developed that is capable of greatly facilitating the identification of novel genes for which some sequence data are known. The polymerase chain reaction (PCR) has been used to identify novel members of several gene families including those of guanine nucleotide regulatory proteins (8) and protein phosphatases (9). PCR has been used to identify novel tyrosine kinase genes (10), though the primers used in that study were designed from DNA segments contained in all tyrosine kinases, rather than being specifically directed against RTK. It is a continuing goal to identify receptors for growth factors.

40 [0007] The elucidation of the growth factors, as well as their receptors, involved in regulating endothelial cell function is critical for the understanding of how new blood vessels are formed (angiogenesis). Angiogenesis plays a significant role in both normal and pathological events such as embryogenesis, progression of ocular diseases, and wound healing (11). In particular, angiogenesis is an important process for the growth of tumors (11). Angiogenesis is a complex process involving endothelial cell proliferation, migration, and tissue infiltration. These events are stimulated by growth factors which either (i) act directly on endothelial cells (12,13); or (ii) act indirectly by inducing host cells to release specific endothelial cell growth factors (11). One member of the first group is vascular endothelial cell growth factor (VEGF), also known as vascular permeability factor (14-16). Besides its angiogenic activity, VEGF displays the physiological function of increasing the permeability of capillary vessels to different macromolecules (14).

SUMMARY OF THE INVENTION

50 [0008] The present invention relates to novel DNA segments which together comprise a gene which encodes type III RTK. The type III RTK encoded by the gene is designated the KDR protein (which stands for Kinase insert Domain containing Receptor). The KDR protein binds specifically to the growth factor VEGF (vascular endothelial cell growth factor).

55 [0009] The DNA segments are identified and isolated through the use of PCR technology. The overall strategy is summarized as follows:

[0010] PCR is used to amplify the DNA segments corresponding to the kinase insert domains of type III receptor tyrosine kinase genes in an endothelial cell library designated HL10246 (Clontech Laboratories, Inc., Palo Alto, CA). Degenerate oligonucleotide primers are designed which are complementary to conserved tyrosine kinase domains

flanking the kinase insert domains of known type III receptor tyrosine kinases. These primers are used in the PCR procedure. DNA probes, designed from the DNA sequence of the PCR product, are then used to identify cDNA clones of the receptor gene from the original cDNA library.

[0011] In particular, the present invention relates to specific oligonucleotides which, when used as primers for PCR, allow for the amplification of DNA segments corresponding to the kinase insert domains of type III RTK genes.

[0012] In a principal embodiment, the present invention is directed to two of three overlapping DNA segments (designated BTIII081.8, BTIII129.5 and BTIV169) according to claim 1 which comprise the entire coding region of this novel gene, namely, 4,068 nucleotides extending to the 3' end.

[0013] These DNA segments are isolated from a human endothelial cell cDNA library and together comprise the gene coding for a novel type III receptor tyrosine kinase. The human gene containing these DNA segments is referred to hereinafter as KDR (which stands for Kinase insert Domain containing Receptor) or, alternatively, as kdp (which stands for Kinase insert Domain containing Protein). The use of the term KDR is intended to include any DNA segments which form the human gene which encodes the novel type III RTK of this application.

[0014] The DNA segments embodied in this invention are isolated from human sources. The present invention comprises DNA segments, and methods for using these DNA segments, which allow for the identification of a closely related gene in mouse DNA. The methods developed in this invention can be readily used by those skilled in the art for the identification and isolation of closely-related homologues in other species. Therefore, the present invention also embodies all DNA segments from species other than human which encode proteins having substantially the same amino acid sequence as that encoded by the kdp gene.

[0015] The present invention further relates to methods developed for the detection of mRNA's produced as a result of transcription of the sense strands of the DNA segments of this invention. Messenger RNA prepared from bovine endothelial cells are used in developing these methods. The ability to detect mRNA for a novel RTK may ultimately have medical benefit, especially in light of recent observations that the mRNA for certain RTKs are overexpressed in some cancers (5).

[0016] The methods developed in the present invention for detecting mRNA expressed by the kdp gene can be readily used by those of ordinary skill in the art for the detection of mRNA species related to the kdp gene in any cell type and from any species. For this reason, the present invention embodies all mRNA segments which are the result of transcription of the kdp gene.

[0017] The present invention relates to methods for expression of the receptor protein, for example, in CMT-3 cells of monkey kidney origin. The receptor protein, portions thereof, and mutated forms of the receptor protein may be expressed in many other cells by those skilled in the art using methods similar to those described in this application. For this reason, the present invention embodies all proteins encoded by the human KDR gene and proteins encoded by related genes found in other species.

[0018] The present invention further relates to methods for studying the interaction of VEGF to the expressed KDR protein. Recent work in the literature (17) indicates that VEGF is one member of a family of related proteins, and the interaction of growth factors similar to VEGF with the KDR protein can readily be studied by those skilled in the art using methods similar to those described in this application. These methods can readily be modified to study the interaction of candidate pharmaceuticals with the KDR protein towards the goal of developing an antagonist or agonist of VEGF action. For this reason, the present invention embodies methods for studying the interaction of VEGF and VEGF-related growth factors with the KDR protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 depicts a schematic representation of three receptor tyrosine kinase subclasses (6). KI is kinase insert domain; PTK is kinase domain; cys is cysteine rich region.

[0020] Figure 2 depicts the two sets of primers used for PCR (SEQ ID NO: 1 and 2). The nucleotide sequences in appropriate regions of the four known type III receptor tyrosine kinase cDNAs are aligned and degenerate oligonucleotide primers are designed based upon the consensus sequences.

[0021] Figure 3 depicts the amplification of the kinase insert domains using PCR. DNA segments encoding the kinase insert domains of type III receptor tyrosine kinases are amplified by PCR. A sample (5 μ l) is run on a 1.0% agarose gel which is stained with ethidium bromide. DNA size standards (123 bp ladder, Bethesda Research Laboratories, Bethesda, MD) are run as well.

[0022] Figure 4 depicts the DNA sequence of the two PCR products (Panel A: 363 bp segment derived from the 420 bp product (SEQ ID NO: 3); Panel B: 251 bp product (SEQ ID NO: 4)). The two products are purified by agarose gel electrophoresis, digested with SalI and EcoRI, and cloned into the plasmid vector pBlueScribe(+)TM (Stratagene; San Diego, CA). The 420 bp PCR product is digested to 363 bp during this procedure. The DNA sequences for the primers used in the amplification are underlined.

[0023] Figure 5A depicts a computer assisted comparison of the DNA sequence for the 363 bp DNA segment derived

from the 420 bp PCR product with the sequence of a DNA segment of the PDGF receptor (SEQ ID NO: 5) (18). A region of strong homology between the 363 bp segment derived from the 420 bp PCR product and the PDGF receptor is contained in a box. Figure 5B depicts a computer assisted comparison of the DNA sequence for the 251 bp PCR product with the sequence of a DNA segment of the FGF receptor (SEQ ID NO: 6) (7).

[0024] Figure 6 depicts the strategy used for sequencing the insert portions of clones BTIII081.8 and BTIII129.5 and BTIV169. The sequencing reaction uses either synthetic oligonucleotides (represented by boxes at the start of an arrow), or the M13 universal primer (no box) to initiate the reaction. In some cases, portions of these DNA segments are isolated using the restriction enzymes indicated in the figure, and subcloned back into the plasmid vector pUC118, so that the M13 universal primer can be used. The position of the stop codon in BTIII129.5 is indicated. The coding portions of these DNA segments are shown at the bottom of the figure. The relative positions of the 1) membrane spanning portion, 2) kinase domains, and 3) kinase insert domain are indicated. The position of these structural features within the KDR derived DNA segments is compared in relation to their position in the PDGF-receptor ("PDGF-R").

[0025] Figure 7 depicts the DNA and predicted amino acid sequence of KDR, plus the stop codon (nucleotides 1-4071 of SEQ ID NO. 7). The sequence of the DNA segment amplified by PCR is underlined (nucleotides 2749-3105 of SEQ ID NO. 7). Cysteine residues in the putative extracellular domain are circled. Potential N-linked glycosylation sites are indicated by an asterisk. The putative membrane spanning region is enclosed in a box (nucleotides 2293-2367 of SEQ ID NO. 7).

[0026] Figure 8 depicts a hydropathy plot of the predicted amino acid sequence for the KDR protein.

[0027] Figure 9 depicts a comparison of the predicted amino acid sequence in the putative intracellular portion of the KDR protein to the ckit proto-oncogene (SEQ ID No: 8) (3), the CSF-1 receptor (SEQ ID NO: 9) (4), and the PDGF receptor (SEQ ID NO: 10) (18). Exact matches are indicated by an asterisk. Gaps are introduced to achieve maximum alignment. The putative ATP recognition site is indicated by three asterisks.

[0028] Figure 10 depicts the identification of kdp receptor mRNA by Northern blot analysis. Five micrograms of bovine aortic endothelial cell polyA+ RNA are used. A nick-translated [³²P] CTP-labelled EcoRI/BamHI DNA segment (nucleotides 1510-2417 of SEQ ID NO. 7) is used as a probe. Autoradiography is for 36 hours.

[0029] Figure 11 depicts the kdp gene in human and mouse DNA by Southern blot analysis. A nick translated [³²P] CTP-labelled EcoRI/BamHI DNA segment (nucleotides 1510-2417 of SEQ ID NO. 7) is used as the probe. The probe is hybridized to Southern blots containing EcoRI digested DNA from human (lane 1), mouse (lane 2), and human-mouse hybrid cells (19) (lanes 3 and 4). The DNA used in lane 3 lacks the kdp locus, while DNA used in lane 4 contains the kdp locus.

[0030] Figure 12 depicts a Western blot analysis of CMT-3 cells which express the KDR protein. Cells are transfected with either the pcDNAItkpASP vector alone (lane 1) or with that vector modified to contain the KDR gene (lane 2). 2 x 10⁵ cells and 1 microgram of DNA are used for each transfection. Forty-eight hours later, Western blot analysis is performed on the samples using the anti-KDR.PS23 polyclonal antibody at a dilution of 1:1000. Detection of reacting proteins is performed using an ECL system (Amersham, Chicago, IL).

[0031] Figure 13 depicts the results of [¹²⁵I] VEGF binding to CMT-3 cells which express the KDR protein. Cells are transfected with either the vector alone (bars 1 and 2) or with the vector containing the KDR gene (bars 3 and 4). Forty-eight hours later, the samples are washed with phosphate buffered saline (PBS), and incubated with serum-free media containing 50 pM [¹²⁵I] VEGF (specific activity equal to 4,000 cpm per fmol), for 90 minutes. Nonradioactive VEGF, 5 nM, is added to some samples (bars 2 and 4) to define specific binding sites. The samples are washed with ice cold PBS, and the cells are transferred to gamma-counting tubes using 0.1% lubrol.

[0032] Figure 14 depicts the results of affinity cross-linking of [¹²⁵I] VEGF to CMT-3 cells which express the KDR protein. CMT-3 cells are transfected with either the vector alone (lane 1) or with the vector containing the KDR gene (lane 2). Forty-eight hours later, the cells are washed in PBS, and serum free media containing 200 pM [¹²⁵I] VEGF is added. After 90 minutes at room temperature, an affinity cross-linker disuccinimidyl suberate, 0.5 mM, is added for 15 minutes. The samples are then prepared for SDS-PAGE autoradiography.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The strategy used to discover the DNA segments for the novel type III RTK gene begins with the design of two degenerate oligonucleotide primers based upon their homology to specific regions of the kinase domains of known RTK genes (Fig. 2) (3,4,7,18). In one embodiment, the polymerase chain reaction is then used to amplify DNA segments from a human endothelial cell cDNA library (designated HL 10246). The cDNA products from this step are each cloned into a plasmid vector designated pBlueScribe(+)TM (Stratagene, San Diego, CA) and sequenced. Oligonucleotide probes are designed from potentially interesting sequences in order to screen the cDNA library for more full length clones of the novel cDNA.

[0034] The strategy just described provides several novel elements: 1) the DNA sequences of the oligonucleotide primers used during PCR; 2) the DNA sequence of the products generated by the polymerase chain reaction; and 3)

the DNA sequence of the final cloned DNA segments. Each of these elements of the invention described in this application will now be discussed in detail.

[0035] Figure 2 shows the rationale for choosing the oligonucleotide primers used in the PCR. The primers are designed to allow for the PCR amplification of the kinase insert domain of type III RTK genes. In order to design the primers, the DNA sequences of known type III RTK genes are aligned in specific regions of their catalytic domains, and a consensus sequence is chosen. The regions of the catalytic domains chosen in designing the primers flank the kinase insert domains of the receptor genes.

[0036] Primer 1 (SEQ ID No: 1) is designed from a region of the kinase domain 5' to the kinase insert domain, and consists of a mixture of four different 21mers. Primer 2 (SEQ ID NO: 2) is designed from a region of the kinase domain 3' to the kinase insert domain, and consists of a mixture of sixteen different 29mers with one inosine, indicated in SEQ ID NO: 2 by "N".

[0037] Sall and EcoRI restriction sites are included at the 5' end of primers 1 and 2, respectively, to facilitate the subcloning of the amplified PCR products into plasmid vectors. Those skilled in the art may use other restriction sites; other minor modifications in the protocol above permits the design of primers without the inclusion of restriction sites.

[0038] The selection of these specific primers constitutes a novel approach towards identifying novel type III RTK genes. It had previously been shown (10) that primers designed from DNA sequences common to all tyrosine kinases allows for the identification of novel proteins. The present invention is the first to contemplate the use of PCR to specifically target type III RTK.

[0039] The protocol used for PCR is as follows: Human endothelial cell cDNA (designated HL10246) is denatured by boiling and submitted to 30 cycles of PCR using 1 nmol of both primers in a final volume of 100 μ l. The timing is 1.5 minutes at 92°C, 2 minutes at 50°C, and 2 minutes at 74°C. DNA from 5 μ l of sample is separated on a 1% agarose gel and stained with ethidium bromide.

[0040] Figure 3 shows the results of the PCR amplification. Two DNA products, with sizes 251 bp (SEQ ID NO: 4) and 420 bp, are visible when a sample of the reaction is electrophoresed on a 1.0% agarose gel and stained with ethidium bromide. The sizes of the two products are within the range expected for type III RTK genes (products derived from the FGF and PDGF receptor genes, which have the smallest and largest known kinase insert domains, would be 230 and 510 bp, respectively (20, 21).

[0041] The DNA from four contiguous lanes with sizes ranging from 200 to 600 bp is electrophoresed onto DEAE filter paper, eluted from the paper with salt, and ethanol precipitated. The samples are incubated with 5 units of EcoRI and Sall. The restriction enzymes digest the 420 bp DNA segment to a 363 bp DNA segment (SEQ ID NO: 3), due to the presence of an EcoRI site within the 420 bp DNA segment (nucleotide 2749, SEQ ID NO: 7). The restriction enzyme digested PCR products are then subcloned into the plasmid vector pBlueScribe(+)TM. The recombinant clones are analyzed by sequencing using the dideoxy-method (22) using a United States Biochemical (Cleveland, Ohio) Sequenase Version 2.0 sequencing kit. Figure 4 shows the DNA sequences for the 251 bp PCR product and the 363 bp DNA segment derived from the 420 bp PCR product.

[0042] Computer assisted comparison of the DNA sequence for the 363 bp segment of the 420 bp PCR product to databases of known DNA sequences reveals that the sequence is novel, because it shares strong sequence identity with the flanking catalytic domain of known type III RTK genes, but not their kinase insert domains. Figure 5A compares the DNA sequence for the 363 DNA segment with that for the PDGF receptor gene (SEQ ID No: 5). Similar results are obtained using other type III RTK genes.

[0043] DNA sequencing of the 251 bp PCR product reveals a novel sequence containing both primers used for the amplification, but the sequence shows little homology to known tyrosine kinases. This is depicted in Figure 5B, which compares the DNA sequence for the 251 bp DNA segment with that for the FGF receptor (SEQ ID NO: 6). For this reason, further analysis of Product 1 is not pursued.

[0044] The protocols used during the PCR do not allow for amplification of the kinase insert domains of known receptor tyrosine kinases in the endothelial cell library used because of the low copy number of the message present in the library. There have been many studies on the effect of FGF on endothelial cell function (23,24) although there is evidence that the expression of the FGF receptor is developmentally regulated (7) and it is likely that the library used contains little or no cDNA for the FGF receptor.

[0045] An oligonucleotide probe, designed from the DNA sequence of the 363 bp segment, is synthesized (using an ABI 380 DNA Synthesizer) in order to screen the human endothelial cell cDNA library (HL10246) for the isolation of more full length clones containing the 363 bp DNA segment. The probe sequence is chosen from the region of the 363 bp DNA segment which shares little sequence homology with known RTK.

[0046] The screening of the endothelial cell cDNA library is conducted as follows: Lambda gt11 phage, 10⁶, are adsorbed to E. coli LE392 for 15 minutes at 37°C prior to plating onto agar plates at a density of 5 x 10⁵ phage per plate. After allowing the phage plaques to develop at 37°C, plaque lifts are made using nitrocellulose filters, denatured in 0.4 N NaCl for 1 minute, and neutralized in 0.5 M Tris.HCl, pH 7.3, plus 1.5 M NaCl. The filters are washed with 2 x standard saline citrate (SSC) and then baked for 1.5 hour in a vacuum oven at 80°C. The filters are probed with an

[³²P] ATP end labeled synthetic oligonucleotide, 5'-TTTCCCTTGACGGAATCGTGCCCCCTTTGGT-3', which is the reverse complement of a DNA sequence contained in the PCR amplified product (Fig. 3). Hybridization is performed at 50°C in 5 x SSPE (167 mM NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA), 2.5 x Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg/ml salmon sperm DNA. The filters are washed twice, 20 minutes per wash, with 2 x SSC plus 0.1% SDS at room temperature, followed by washing twice at 50°C with 0.1 X SSC plus 0.1% SDS; 20 minutes per wash. Positive clones are identified, picked and plaque purified.

[0047] Forty-five positive clones are obtained. Three of these positive clones are plaque purified and their phage DNA isolated. Digestion of the DNA with EcoRI and electrophoresis in agarose indicates that one clone, designated BTIII081.8, contains the largest insert, and subsequent analysis indicates that the DNA insert of this clone overlaps that of the inserts contained in other two purified clones (designated BTIII079.11 and BTIII079.47A).

[0048] Digestion of the purified phage DNA of the clone designated BTIII081.8 with EcoRI results in DNA segments of 250 bp, 600 bp, and 1000 bp. Each of these three products is subcloned into the plasmid vector pUC118 and sequenced (Figure 6 shows the strategy used for sequencing). The orientation of the three fragments is determined by subcloning from the insert a BglII/BglII fragment into pUC118 and sequencing across the EcoRI junctions using a synthetic oligonucleotide to prime the sequencing reaction.

[0049] A restriction map is determined for each fragment (Figure 6). Various restriction site pieces are removed from the plasmids and recloned into pUC118 so that sequencing the resulting plasmids with the universal primer allows for sequencing most of the entire original fragments in both directions. Three oligonucleotide primers are required to sequence the entire cDNA in both directions. For the purposes of this application, this insert contains nucleotides numbered 1510-3406 (SEQ ID NO. 7).

[0050] A [³²P]CTP-labelled, nick-translated EcoRI-BamHI DNA segment derived from clone BTIII081.8 (nucleotides 1510-2417 of SEQ ID NO. 7) is used as a probe to rescreen the original endothelial cell cDNA library for more 5' full length DNA segments of the gene from which the insert portion of BTIII081.8 is derived. The protocols used to isolate the overlapping clones are identical to that used to isolate BTIII081.8.

[0051] A synthetic oligonucleotide probe is designed with 29 nucleotides corresponding to part of the DNA sequence of the insert portion of the clone BTIII081.8 (nucleotides 3297-3325 of SEQ ID NO. 7) in order to rescreen the original endothelial cell cDNA library for more full 3' length DNA segments of the gene from which the insert portion of BTIII081.8 is derived. The protocols used to isolate the overlapping clones are identical to that used to isolate BTIII081.8. Several positive clones for each of the 5' and 3' ends are identified and plaque purified.

[0052] One of the clones is designated BTIII200.2. The DNA from BTIII200.2 contains a 3.4 kb insert as determined by EcoRI digestion of the isolated phage DNA. EcoRI digestion of BTIII200.2 results in three DNA fragments. One of these fragments (2.5 kb) is cloned into pUC119 and is designated BTIV006. The clone BTIV006 contains nucleotides numbered 7-2482. As described below, BTIV006 plus nucleotides 1-6 is designated BTIV169. DNA sequencing of the 2.5 kb DNA insert (BTIV169) indicates that it overlaps over one thousand nucleotides of the DNA sequence of the insert portion of the clone BTIII081.8 (Figure 6) at the 5' end.

[0053] A second clone isolated from the cDNA library is designated BTIII129.5. The DNA from BTIII129.5 contains a 2.2 kb insert as determined by EcoRI digestion of the isolated phage DNA. DNA sequencing of the 2.2 kb DNA insert indicates that it overlaps over five hundred nucleotides of the DNA sequence of the insert portion of the clone BTIII081.8 (Figure 6). The clone BTIII129.5 contains nucleotides numbered 2848-4236 (SEQ ID NO. 7). The DNA sequence for BTIII129.5 contains the stop codon TAA, defining the position of the 3' end of an open reading frame for the novel gene. Except for the first six nucleotides of the gene which are discussed below, these three clones define a gene encoding a growth factor receptor. These three clones define a 4,062 nucleotide sequence of the open reading frame of the gene extending to the 3' end, followed by a 168 nucleotide non-coding region (SEQ ID NO. 7). A sample of a lambda gt11 phage harboring the clone BTIII081.8 has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A., and has been assigned ATCC accession number 40,931. A sample of a lambda gt11 phage harboring the clone BTIII129.5 has been deposited with the American Type Culture Collection and has been assigned ATCC accession number 40,975. For reasons discussed below, a sample of the clone BTIV006 was not deposited.

[0054] The aforementioned DNA segments (BTIII081.8, BTIII129.5, and BTIII200.2 (or BTIV006) encode 4062 nucleotides of the coding portion of a novel gene. The cDNA clones are incomplete in that a transcription initiation coding for methionine is missing. After the isolation of these clones, Matthews et al. (25) reported the cloning of a gene homologue of KDR in mouse, which was referred to as Flk-1. Analysis of the nucleic acid and amino acid sequence of Flk-1 indicated that the addition of six nucleotides to the 5' end of the isolated KDR clones would provide for a complete coding region.

[0055] To achieve this, an EcoRI-BamHI restriction fragment of BTIV200.2 is cloned into the plasmid pBlueScript KS™ (Stratagene, La Jolla, CA). The 5' end of the inserted DNA is blunt ended with Kienow polymerase and Mung Bean nuclease. Next, the synthetic oligonucleotide TCGACGCGCG ATG GAG (SEQ ID NO. 11) is cloned into this vector. The oligonucleotide contains the sequence ATG GAG in frame with the downstream DNA insert. These nucle-

otides (ATG GAG) encode the amino acids methionine and glutamic acid, the first two amino acids encoded by the KDR gene. The resulting plasmid vector is designated BTIV140. This plasmid is purified on a CsCl gradient.

[0056] The purified plasmid is designated BTIV169. The insert of BTIV169 contains nucleotides 1-2400 (SEQ ID NO. 7) of the KDR gene. A sample of the plasmid pBlueScript KS™ which contains the clone BTIV169 has been deposited

with the American Type Culture Collection and has been assigned ATCC accession number 75200.

[0057] Thus, together the clones BTIII081.8, BTIII129.5 and BTIV169 comprise the entire open reading frame of 4,068 nucleotides for the novel KDR gene. As will be discussed below, the KDR gene expresses the novel KDR receptor which binds specifically to the growth factor VEGF.

[0058] DNA sequencing of BTIII081.8, BTIII129.5 and BTIV169 (SEQ ID NO. 7) shows that the newly isolated gene is similar to, but distinct from, previously identified type III RTK. The predicted amino acid sequence (SEQ ID NO. 7) contains several structural features which demonstrate that the novel gene is a type III RTK. These structural features are summarized as follows:

1) A hydropathy plot of the predicted amino acid sequence indicates a single membrane spanning region (see Figure 8). This is characteristic of a type III RTK (Figure 7).

2) The putative amino-terminal 762 amino acid portion of the receptor has structural features of extracellular receptor ligand binding domains (1), including regularly spaced cysteines and 18 potential N-linked glycosylation sites (Figure 7).

3) The predicted amino acid sequence of the carboxy-terminal 530 amino acid portion contains an ATP-binding site at lysine 868, 22 amino acids downstream from the consensus ATP recognition sequence Gly-X-Gly-X-X-Gly (26) (Figure 8).

4) Within the kinase domain there is a 55-60% identical match in amino acid sequence to three other type III receptor tyrosine kinases: ckit proto-oncogene (SEQ ID NO: 8), CSF-1 (SEQ ID NO: 9) and PDGF (SEQ ID NO: 10) (Figure 9).

5) The predicted kinase domain contains a kinase insert domain of approximately 71 amino acids. As indicated in Figure 9, this portion of the amino acid sequence shares little sequence homology with other type III RTK.

[0059] The endothelial cell library can be further screened to isolate the 5' untranslated region and genomic clones can be generated so as to isolate the promoter region for the KDR gene.

[0060] In addition to the DNA sequence described for the KDR gene (SEQ ID NO. 7), the present invention further comprises DNA sequences which, by virtue of the redundancy of the genetic code, are biologically equivalent to the sequences which encode for the receptor, that is, these other DNA sequences are characterized by nucleotide sequences which differ from those set forth herein, but which encode a receptor having the same amino acid sequences as those encoded by the DNA sequences set forth herein.

[0061] In particular, the invention contemplates those DNA sequences according to claim 1 which are sufficiently duplicative of the sequence of SEQ ID NO. 7 so as to permit hybridization therewith under standard high stringency Southern hybridization conditions, such as those described in Sambrook et al. (27), as well as the biologically active proteins produced thereby.

[0062] This invention also comprises DNA sequences according to claim 1 which encode amino acid sequences which differ from those of the novel receptor, but which are the biological equivalent to those described for the receptor. Such amino acid sequences may be said to be biologically equivalent to those of the receptor if their sequences differ only by minor deletions from or conservative substitutions to the receptor sequence, such that the tertiary configurations of the sequences are essentially unchanged from those of the receptor.

[0063] For example, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, as well as changes based on similarities of residues in their hydropathic index, can also be expected to produce a biologically equivalent product. Nucleotide changes which result in alteration of the N-terminal or C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. It may also be desirable to eliminate one or more of the cysteines present in the sequence, as the presence of cysteines may result in the undesirable formation of multimers when the protein is produced recombinantly, thereby complicating the purification and crystallization processes. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Therefore, where the terms "KDR gene" or "KDR-protein" are used in either the specification or the claims, each will be understood to encompass all such modifications and variations which result in the production of a biologically equivalent protein.

[0064] To determine the size of the mRNA transcribed from the kdp gene, Northern blot hybridization experiments are carried out using an EcoRI/BamHI DNA segment (nucleotides 1510-2417, SEQ ID NO. 7) as a hybridization probe.

The DNA used for the probe does not contain any portion of the putative kinase domain, and shares little sequence homology to other tyrosine kinases. The Northern blot analysis (Figure 10) shows that a 7 kb band is visualized in cytoplasmic poly(A)+ RNA of ABAE bovine aortic endothelial cells. This transcript differs in size from previously reported transcripts for known type III RTK (7,18).

[0065] The isolated cDNA is significant for several reasons. The cDNA encodes a novel type III receptor tyrosine kinase. The homology between the sequence of this cDNA and that of other receptors, as well as structural properties implied by the predicted amino acid sequence confirm the relationship. Receptors for growth factors should have tremendous utility in drug development as they face the outside of the cell and thus are among the best targets for drugs. In addition, the cellular levels of some receptors, in particular the neu proto-oncogene, increase during some cancers. This has been taken advantage of in designing diagnostic tests for these cancers.

[0066] Southern analysis demonstrates that the kdp gene is present in mouse as well as human DNA. Mouse and human (Hela cell) DNA, 15 µg of each, are digested with 10 units of *EcoRI* and electrophoresed on a 0.7% agarose gel. The DNA is transferred onto nitrocellulose. The filter is hybridized to a [³²P]CTP-labelled cDNA probe made by nick translating an *EcoRI*/*Bam*HI fragment from the 5' end of the kdp cDNA (nucleotides 1510-2417, SEQ ID NO. 7). Hybridization is conducted at 30°C in 5 X SSPE, 50% formamide, 0.1% SDS, plus 150 µg/ml salmon sperm DNA. The DNA probe hybridizes to Southern blots containing *EcoRI* digested DNA. After 48 hours, the filter is washed at room temperature in 2 X SSC plus 0.1% SDS for 20 minutes, followed by two 20 minute washes at 40°C with 0.1 X SSC plus 0.1% SDS. Autoradiography is then performed for 48 hours. As shown in Figure 11, radioactively labelled DNA is present in both human and mouse samples. This indicates that the kdp gene is present in both species.

[0067] An experiment is conducted to ascertain the genetic locus of kdp on human chromosomes. Thirty-eight cell hybrids from 18 unrelated human cell lines and four mouse cell lines are examined (19). A DNA probe hybridizes to Southern blots which contain *EcoRI* digested DNA from the human-mouse hybrids (using the procedure and DNA probe for human and mouse tissue described in relation to Figure 11). Table I sets forth the results of the segregation of kdp with human chromosomes in *EcoRI* digested human-mouse somatic cell hybrid DNA:

Table I

Chromosome	Concordant # of Hybrids (+/+) (-/-)		Discordant # of Hybrids (+/-) (-/+)		% Discordancy
1	4	19	8	4	34
2	8	18	5	6	30
3	11	12	3	9	34
4	14	24	0	0	0
5	7	14	7	10	45
6	7	19	7	5	32
7	11	14	3	8	31
8	8	11	6	13	50
9	3	20	10	4	38
10	12	9	2	14	43
11	9	13	4	11	41
12	9	10	5	14	50
13	7	18	7	6	34
14	11	8	3	16	50
15	9	15	5	8	35
16	7	19	7	5	32
17	12	7	2	16	49
18	11	14	3	10	34
19	7	18	7	6	34
20	9	10	5	14	50
21	11	9	3	15	47
22	3	16	10	7	47
X	8	10	3	8	38

[0068] The scoring is determined by the presence(+) or absence (-) of human bands in the hybrids on Southern blots prepared in a similar to those shown in Figure 11. The scoring is compared to the presence or absence of human chromosomes in each hybrid. A 0% discordancy indicates a matched segregation of the DNA probe with a chromosome.

Three fragments, approximately 6.5 kb, 3.1 kb, and 0.7 kb in size are detected in digests of human DNA (Figure 11), and in all hybrids which had retained human chromosome 4 (Table I). All other chromosomes are excluded in at least 11 discordant hybrids (Table I). The results of Figure 11 and Table I demonstrate that the genetic locus of kdp is on human chromosome 4.

[0069] It is noteworthy that both the *ckit* (3) and the type A PDGF (28) receptor genes map to human chromosome 4. The finding that the genetic locus of kdp is on human chromosome 4 provides further evidence that the novel receptor of this invention is a type III receptor tyrosine kinase.

[0070] The next step after identifying the entire coding portion of the kdp gene is to express the receptor protein encoded by that gene. The receptor protein is then utilized so as to identify the growth factor which binds specifically to the receptor.

[0071] The receptor protein is expressed using established recombinant DNA methods. Suitable host organisms include bacteria, viruses, yeast, insect or mammalian cell lines, as well as other conventional organisms. For example, CMT-3 monkey kidney cells are transfected with a vector containing the complete coding region of the *KDR* gene.

[0072] The complete coding portion of the *KDR* gene is assembled by sequentially cloning into pUC119 three DNA fragments derived from BTIII081.8, BTIII129.5, and BTIV169. First, a *Sma*I-*Eco*RI fragment of clone BTIII129.5 (nucleotides 3152-4236, SEQ ID NO: 7) is blunt ended with Klenow polymerase and introduced into a *Sma*I site in pUC119. Next, a *Bam*HI-*Sma*I fragment of clone BTIII081.8 (nucleotides 2418-3151, SEQ ID NO: 7) is introduced at a *Bam*HI-*Sma*I site. Finally, a *Sall*-*Bam*HI fragment of clone BTIV169 (nucleotides 1-2417, SEQ ID NO: 7) is introduced at a *Sall*-*Bam*HI site. Part of the cloning site of pUC119 is contained in the *Sall*-*Bam*HI fragment, 5' to the *KDR* gene. In order to clone the complete coding portion into an expression vector, the assembled DNA (in pUC119) is digested with *Sall* and *Asp*118 and recloned into the eukaryotic expression vector pcDNAItkpASP.

[0073] This vector is a modification of the vector pcDNAI (Invitrogen; San Diego, CA). Specifically, the ampicillin resistance gene is cloned from pBR322 into pcDNAI. A small SV40 T splice and the SV40 polyadenylation signal are then removed and are replaced with a Herpes Simplex Virus-1 polyadenylation signal. Finally, a cytomegalovirus intermediate early splice is inserted 5' to the cloning site to yield pcDNAItkpASP.

[0074] Transfection of CMT-3 cells is done using DEAE-dextran. Forty-eight hours after transfection, expression of the novel receptor is monitored using Western blot analysis as follows.

[0075] An antibody is used to assay the expressed receptor protein. The predicted amino acid sequence of the receptor is used to generate peptide-derived antibodies to the receptor by conventional techniques. The presence of the novel receptor protein is confirmed by Western blot hybridization.

[0076] Specifically, a synthetic peptide with 13 residues is synthesized based on the 12 residues corresponding to amino acids 986-997 of the putative amino acid sequence of the *KDR* protein (SEQ ID NO: 7), with a cysteine residue linked to the lysine (amino acid 997). The cysteine facilitates coupling of the peptide to a macromolecule which functions as a carrier for the peptide. For example, the peptide is coupled to keyhole limpet haemocyanin (KLH) using m-maleimidobenzoyl-N-hydroxysuccinimide ester. Other conventional carriers may be used such as human and bovine serum albumins, myoglobins, β -galactosidase, penicillinase and bacterial toxoids, as well as synthetic molecules such as multi-poly-DL-alanyl-poly-L-lysine and poly-L-lysine.

[0077] Rabbits are immunized with the peptide-KLH conjugate to raise polyclonal antibodies. After different periods of time, serum is collected from the rabbits. The IgG fraction of the serum is then purified using a protein A Sepharose column (Pharmacia LKB, Uppsala, Sweden) to obtain the antibody which is designated anti-*KDR*.PS23.

[0078] A sample of the expressed *KDR* protein is subjected to SDS-PAGE using a 7% acrylamide gel under standard conditions. The protein band is then transferred onto nitrocellulose paper for Western blot analysis and the anti-*KDR*.PS23 antibody is added at a dilution of 1:1,000 to allow the antibody to react with the protein present. A second antibody, goat anti-rabbit antibody to rabbit IgG, which binds to anti-*KDR*.PS23, is then added. The detection of proteins which react with the antibodies is performed by autoradiography of bands using an ECL system (Amersham, Chicago, IL). The results are depicted in Figure 12.

[0079] Figure 12 shows that a 190 kD protein is present in the cells transfected with the vector containing the *KDR* gene, but is absent in cells transfected with vector alone. The size of this protein is consistent with it being encoded by the *KDR* gene, in that the predicted amino acid sequence for the unglycosylated *KDR* protein is 156 kD, and that sequence contains 18 putative extracellular glycosylation sites which would account for the balance of the size seen in the 190 kD band.

[0080] The expressed receptor is then used to identify the growth factor which interacts with the receptor. In order to test the hypothesis that the *KDR* protein is a receptor for VEGF, radioligand binding studies are performed. VEGF (provided by D. Gospodarowicz) is radiolabelled with 125 I. Cells are transfected with either the vector pcDNAItkpASP alone (bars 1 and 2 of Figure 13) or with the vector containing the *KDR* gene (bars 3 and 4). Forty-eight hours later, the transfected cell samples are washed with PBS and then incubated for 90 minutes with serum-free media containing 50 pM [125 I]VEGF (specific activity equal to 4,000 cpm per fmol). Excess nonradioactive VEGF, 5 nM, is added to some samples (bars 2 and 4) to define specific binding sites. The samples are washed with ice cold PBS, and the cells are

transferred to gamma-counting tubes using a detergent, 0.1% lubrol.

[0081] The results of the radioligand binding studies are depicted in Figure 13. Figure 13 shows that CMT-3 cells transfected with vector containing the KDR gene contain specific binding sites for [¹²⁵I]VEGF (compare bars 3 and 4), while cells transfected with vector alone do not (compare bars 1 and 2).

[0082] Further evidence that the KDR gene encodes a receptor for VEGF is demonstrated by affinity cross-linking studies (Figure 14). Figure 14 depicts the results of affinity cross-linking of [¹²⁵I]VEGF to CMT-3 cells which express the KDR protein. CMT-3 cells are transfected with either the pcDNA1tkpASP vector alone (lane 1 of Figure 14) or with the vector containing the KDR gene (lane 2). Forty-eight hours later, the cells are washed in PBS, and serum free media containing 200 pM [¹²⁵I]VEGF is added. After 90 minutes at room temperature, an affinity cross-linker disuccinimidyl suberate (Pierce Biochemicals, Rockford, IL), 0.5mM, is added for 15 minutes. The samples are then subjected to SDS-PAGE autoradiography.

[0083] Three protein bands are seen in SDS-PAGE autoradiograms from samples of CMT-3 cells transfected with the KDR gene and cross-linked to [¹²⁵I]VEGF (lane 1). The size of band 1 (235 kD) is consistent with it being the 190 kD protein seen by Western blot analysis (Figure 12), because a 45 kD [¹²⁵I] VEGF dimer plus 190 kD would migrate in a manner identical to band 1. The origin of band 2 is not clear, but may represent an altered glycosylation form of band 1. Band 3 (22.5 kD) is most likely VEGF itself, and can be seen faintly in cells transfected with vector alone (lane 2).

[0084] The novel KDR gene of this invention is significant for several reasons. Studies of the cellular mechanisms by which receptors function in signal transduction have led in the past to a better understanding of how cells grow in both normal and diseased states. Receptor tyrosine kinases, in particular, have received a great deal of attention because of the observation that a number of RTK are the cellular counterparts for viral oncogenes, implying a direct correlation between changes in the expression of RTK and cancer. In view of this, it is likely that pharmaceuticals targeted at RTK will inhibit the changes in cell growth associated with cancer. In addition, it is likely that monitoring the levels of expression of RTK will prove valuable in diagnosing the onset of cancer.

[0085] The described cDNA is isolated from a human endothelial cell library. Endothelial cells participate in angiogenesis, the formation of new blood capillaries. Previous work directed towards identifying the growth factors which regulate angiogenesis have primarily focused upon FGF (13), although recent evidence has indicated that other growth factors may be involved as well (12,15,29). This evidence consists of the observations that: 1) FGF does not contain a signal sequence (24) and thus may not be secreted from cells in a manner consistent with the tight regulation of angiogenesis, and 2) endothelial cells synthesize FGF and yet are normally resting (15). Our discovery, then, of a novel growth factor receptor may ultimately clarify these inconsistencies and lead to a better understanding of endothelial cell function.

[0086] The teachings of this invention can be readily used by those skilled the art for the purpose of testing pharmaceuticals targeted at the KDR protein. Two examples of approaches which can be used for this purpose are now given.

[0087] First, the methods described in this invention for studying the interaction of VEGF with KDR protein can be used to test for pharmaceuticals which will antagonize that interaction. For these studies, cells expressing the KDR protein are incubated with [¹²⁵I]VEGF, together with a candidate pharmaceutical. Inhibition of radioligand binding is tested for; significant inhibition indicates the candidate is an antagonist. Permanent expression of the KDR protein in a cell type such as NIH3T3 cells would make these studies less laborious. This can be easily achieved by those skilled in the art using the described methods.

[0088] Second, using the teachings of this invention, those skilled in the art can study structural properties of the KDR protein involved in receptor function. This structural information can then be used to more rationally design pharmaceuticals which inhibit that function. Mutagenesis of the KDR gene by well established protocols is one approach, crystallization of the receptor binding site is another.

Bibliography

[0089]

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SEQUENCE LISTING

[0090]

(1) GENERAL INFORMATION:

(i) APPLICANT: Terman, Bruce I
Carrion, Miguel E

(ii) TITLE OF INVENTION: Identification of a Novel Human Growth Factor Receptor

(iii) NUMBER OF SEQUENCES: 14

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC AT

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: ASCII from IBM DW 4

(vi) CURRENT APPLICATION DATA:

- 5 (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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- 20 (A) TELEPHONE: 203 321 2719
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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

40 GTCGAC AAY CTG TTG GGR GCC TGC AAC 27

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

55 GAATTC AG CAC KTT NCT RGC YGC CAG GTC TGY GTC 35

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 363 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

15 GAA TTC TGC AAA TTT GGA AAC CTG TCC ACT TAC CTG 36
 AGG ACG AAG AGA AAT GAA TTT GTC CCC TAC AAG ACC 72
 20 AAA GGG GCA CGA TTC CGT CAA GGG AAA GAC TAC GTT 108
 GGA GCA ATC CCT GTG GAT CTG AAA CGG CGC TTG GAC 144
 25 ACG CAT CAC CAG TAG CCA GAG CTC AGC CAG CTC TGG 180
 30 ATT TGT GGA GGA GAA GTC CCT CAG TGA TGT AGA AGA 216
 AGA GGA AGC TCC TGA AGA TCT GTA TAA GGA CTT CCT 252
 35 GAC CTT GGA GCA TCT CAT CTG TTA CAG TTT CCA AGT 288
 GGC TAA GGG CAT GGA GTT CTT GGC ATC GCG AAA GTG 324
 40 TAT CCA CAG AGA CCT GGC AGC CAG GAA CGT GCT GAA 360
 45 TTC 363

(2) INFORMATION FOR SEQ ID NO: 4 :

50 (i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 251 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

5
 10
 15
 20

GTC GAC AAT CTG TTG GGG GCC TGC ACC ATC CCA ACA	36
TCC TGC TGC TCT ACA ACT ATT TTT ATG ACC GGA GGA	72
GGA TCT ACT TGA TTC TAG AGT ATG CCC CCC GCG GAG	108
CTC TAC AAG GAG CTG CAG AAG AGC TGC ACA TTT GAC	144
GAG CAG CGA ACA GCC ACG ATC ATG GAG GAG TTG GCA	180
GAT GCT CTA ATG TAC TGC CGT GGG AAG AAG GTG ATT	216
CAC AGA GAC CTG GCA GCC AGC AAC GTG CTG AAT TC	251

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 510 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: PDGF Receptor DNA
 (B) LOCATION: Internal sequence

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Gronwald, R., et al.
 (B) JOURNAL: Proc. Natl. Acad. Sci.
 (C) VOLUME: 85
 (D) PAGES: 3435-3439
 (E) DATE: 1988

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AAC CTG TGG GGG CCT GCA CCA AAG GAG GAC CAT CTA 36

	TAT CAT CTA TAT CAT CAC TGA GTA CTG CCG CTA CGG	72
5	AGA CCT GGT GGA CTA CCT GCA CCG CAA CAA ACA CAC	108
	CTT CCT GCA GCA CCA CTC CGA CAA GCG CCG CCC GCC	144
10	CAG CGC GGA GCT CTA CAG CAA TGC TCT GCC CGT TGG	180
	GCT CCC CCT GCC CAG CCA TGT GTC CTT GAC CGG GGG	216
15	AGA GCG ACG GTG GCT ACA TGG ACA TGA GCA AGG ACG	252
	AGT CGG TGG ACT ATG TGC CCA TGC TGG ACA TGA AAG	288
20	GAG ACG TCA AAT AGC AGA CAT CGA GTC CTC CAA CTA	324
	CAT GGC CCC TTA CGA TAA CTA CGT TCC CTC TGC CCC	360
25	TGA GAG GAC CTG CCG AGC AAC TTT GAT CAA CGA GTC	396
	TCC AGT GCT AAG CTA CAT GGA CCT CGT GGG CTT CAG	432
30	CTA CCA GGT GGC CAA TGG CAT GGA GTT CTG GCC TCC	468
	AAG AAC TGC GTC CAC AGA GAC CTG GCG GCT AGG AAC	504
35	GTC CTT	510

45

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

50

- (A) LENGTH: 255 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55

- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:

(A) NAME/KEY: FGF Receptor DNA

(B) LOCATION: Internal sequence

(x) PUBLICATION INFORMATION:

- 5 (A) AUTHORS: Ruta, M., et al.
 (B) JOURNAL: Oncogene
 (C) VOLUME: 3
 (D) PAGES: 9-15
 (E) DATE: 1988

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

15 AAC CTG CTG GGG GCC TGC ACG CAG GAT GGT CCC TTG 36
 TAT GTC ATC GTG GAG TAT GCC TCC AAG GGC AAC CTG 72
 20 CGG GAG TAC CTG CAG ACC CGG AGG CCC CCA GGG CTG 108
 GAA TAC TGC TAT AAC CCC AGC CAC AAC CCA GAG GAG 144
 25 CAG CTC TCC TCC AAG GAC CTG GTG TCC TGC GCC TAC 180
 30 CAG GAG GCC CGA GGC ATG GAG TAT CTG GCC TCC AAG 216
 AAG TGC ATA CAC CGA GAC CTG GCA GCC AGG AAT GTC 252
 35 CTG 255

40 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 4236 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

55

	ATG GAG AGC AAG GTG CTG CTG GCC GTC GCC CTG	33
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5	1 5 10	
	TGG CTC TGC GTG GAG ACC CGG GCC GCC TCT GTG GGT	69
10	Trp Leu Cys Val Glu Thr Arg Ala Ala Ser Val Gly	
	15 20	
15	TTG CCT AGT GTT TCT CTT GAT CTG CCC AGG CTC AGC	105
	Leu Pro Ser Val Ser Leu Asp Leu Pro Arg Leu Ser	
	25 30 35	
20		
25		
30		
35		
40		
45		
50		
55		

5 ATA CAA AAA GAC ATA CTT ACA ATT AAG GCT AAT ACA 141
 Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr
 40 45

10 ACT CTT CAA ATT ACT TGC AGG GGA CAG AGG GAC TTG 177
 Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu
 50 55

15 GAC TGG CTT TGG CCC AAT AAT CAG AGT GGC AGT GAG 213
 Asp Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu
 60 65 70

20 CAA AGG GTG GAG GTG ACT GAG TGC AGC GAT GGC CTC 249
 Gln Arg Val Glu Val Thr Glu Cys Ser Asp Gly Leu
 75 80

25 TTC TGT AAG ACA CTC ACA ATT CCA AAA GTG ATC GGA 285
 Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly
 85 90 95

30 AAT GAC ACT GGA GCC TAC AAG TGC TTC TAC CGG GAA 321
 Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu
 100 105

35 ACT GAC TTG GCC TCG GTC ATT TAT GTC TAT GTT CAA 357
 Thr Asp Leu Ala Ser Val Ile Tyr Val Tyr Val Gln
 110 115

40 GAT TAC AGA TCT CCA TTT ATT GCT TCT GTT AGT GAC 393
 Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp
 120 125 130

45 CAA CAT GGA GTC GTG TAC ATT ACT GAG AAC AAA AAC 429
 Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn
 135 140

55

	AAA ACT GTG GTG ATT CCA TGT CTC GGG TCC ATT TCA	465
	Lys Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser	
5	145 150 155	
	AAT CTC AAC GTG TCA CTT TGT GCA AGA TAC CCA GAA	501
10	Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro Glu	
	160 165	
	AAG AGA TTT GTT CCT GAT GGT AAC AGA ATT TCC TGG	537
15	Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp	
	170 175	
	GAC AGC AAG AAG GGC TTT ACT ATT CCC AGC TAC ATG	573
20	Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met	
	180 185 190	
25	ATC AGC TAT GCT GGC ATG GTC TTC TGT GAA GCA AAA	609
	Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys	
30	195 200	
	ATT AAT GAT GAA AGT TAC CAG TCT ATT ATG TAC ATA	645
35	Ile Asn Asp Glu Ser Tyr Gln Ser Ile Met Tyr Ile	
	205 210 215	
	GTT GTC GTT GTA GGG TAT AGG ATT TAT GAT GTG GTT	681
40	Val Val Val Val Gly Tyr Arg Ile Tyr Asp Val Val	
	220 225	
	CTG AGT CCG TCT CAT GGA ATT GAA CTA TCT GTT GGA	717
45	Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly	
	230 235	
50	GAA AAG CTT GTC TTA AAT TGT ACA GCA AGA ACT GAA	753
	Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu	
55	240 245 250	

	CTA AAT GTG GGG ATT GAC TTC AAC TGG GAA TAC CCT	789
	Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro	
5	255 260	
	TCT TCG AAG CAT CAG CAT AAG AAA CTT GTA AAC CGA	825
10	Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg	
	265 270 275	
	GAC CTA AAA ACC CAG TCT GGG AGT GAG ATG AAG AAA	861
15	Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys	
	280 285	
	TTT TTG AGC ACC TTA ACT ATA GAT GGT GTA ACC CGG	897
20	Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg	
	290 295	
	AGT GAC CAA GGA TTG TAC ACC TGT GCA GCA TCC AGT	933
25	Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser	
30	300 305 310	
	GGG CTG ATG ACC AAG AAG AAC AGC ACA TTT GTC AGG	969
35	Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg	
	315 320	
	GTC CAT GAA AAA CCT TTT GTT GCT TTT GGA AGT GGC	1005
40	Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly	
	325 330 335	
	ATG GAA TCT CTG GTG GAA GCC ACG GTG GGG GAG CGT	1041
45	Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg	
	340 345	
	GTC AGA ATC CCT GCG AAG TAC CTT GGT TAC CCA CCC	1077
50	Val Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro	
55	350 355	

5 CCA GAA ATA AAA TGG TAT AAA AAT GGA ATA CCC CTT 1113
 Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu
 360 365 370

10 GAG TCC AAT CAC ACA ATT AAA GCG GGG CAT GTA CTG 1149
 Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu
 375 380

15 ACG ATT ATG GAA GTG AGT GAA AGA GAC ACA GGA AAT 1185
 Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn
 385 390 395

20 TAC ACT GTC ATC CTT ACC AAT CCC ATT TCA AAG GAG 1221
 Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu
 400 405

25 AAG CAG AGC CAT GTG GTC TCT CTG GTT GTG TAT GTC 1257
 Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
 410 415

30 CCA CCC CAG ATT GGT GAG AAA TCT CTA ATC TCT CCT 1293
 Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro
 420 425 430

35 GTG GAT TCC TAC CAG TAC GGC ACC ACT CAA ACG CTG 1329
 Val Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu
 435 440

40 ACA TGT ACG GTC TAT GCC ATT CCT CCC CCG CAT CAC 1365
 Thr Cys Thr Val Tyr Ala Ile Pro Pro Pro His His
 445 450 455

45 ATC CAC TGG TAT TGG CAG TTG GAG GAA GAG TGC GCC 1401
 Ile His Trp Tyr Trp Gln Leu Glu Glu Glu Cys Ala
 460 465

50
 55

AAC GAG CCC AGC CAA GCT GTC TCA GTG ACA AAC CCA 1437
 Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro

470

475

TAC CCT TGT GAA GAA TGG AGA AGT GTG GAG GAC TTC 1473
 Tyr Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe
 480 485 490

CAG GGA GGA AAT AAA ATT GAA GTT AAT AAA AAT CAA 1509
 Gln Gly Gly Asn Lys Ile Glu Val Asn Lys Asn Gln
 495 500

TTT GCT CTA ATT GAA GGA AAA AAC AAA ACT GTA AGT 1545
 Phe Ala Leu Ile Glu Gly Lys Asn Lys Thr Val Ser
 505 510 515

ACC CTT GTT ATC CAA GCG GCA AAT GTG TCA GCT TTG 1581
 Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu
 520 525

TAC AAA TGT GAA GCG GTC AAC AAA GTC GGG AGA GGA 1617
 Tyr Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly
 530 535

GAG AGG GTG ATC TCC TTC CAC GTG ACC AGG GGT CCT 1653
 Glu Arg Val Ile Ser Phe His Val Thr Arg Gly Pro
 540 545 550

GAA ATT ACT TTG CAA CCT GAC ATG CAG CCC ACT GAG 1689
 Glu Ile Thr Leu Gln Pro Asp Met Gln Pro Thr Glu
 555 560

CAG GAG AGC GTG TCT TTG TGG TGC ACT GCA GAC AGA 1725
 Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg
 565 570 575

5 TCT ACG TTT GAG AAC CTC ACA TGG TAC AAG CTT GGC 1761
 Ser Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly
 580 585

10 CCA CAG CCT CTG CCA ATC CAT GTG GGA GAG TTG CCC 1797
 Pro Gln Pro Leu Pro Ile His Val Gly Glu Leu Pro
 590 595

15 ACA CCT GTT TGC AAG AAC TTG GAT ACT CTT TGG AAA 1833
 Thr Pro Val Cys Lys Asn Leu Asp Thr Leu Trp Lys
 600 605 610

20 TTG AAT GCC ACC ATG TTC TCT AAT AGC ACA AAT GAC 1869
 Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp
 615 620

25 ATT TTG ATC ATG GAG CTT AAG AAT GCA TCC TTG CAG 1905
 Ile Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln
 30 625 630 635

35 GAC CAA GGA GAC TAT GTC TGC CTT GCT CAA GAC AGG 1941
 Asp Gln Gly Asp Tyr Val Cys Leu Ala Gln Asp Arg
 640 645

40 AAG ACC AAG AAA AGA CAT TGC GTG GTC AGG CAG CTC 1977
 Lys Thr Lys Lys Arg His Cys Val Val Arg Gln Leu
 650 655

45 ACA GTC CTA GAG CGT GTG GCA CCC ACG ATC ACA GGA 2013
 Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly
 660 665 670

50 AAC CTG GAG AAT CAG ACG ACA AGT ATT GGG GAA AGC 2049
 Asn Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser
 55 675 680

5 ATC GAA GTC TCA TGC ACG GCA TCT GGG AAT CCC CCT 2085
 Ile Glu Val Ser Cys Thr Ala Ser Gly Asn Pro Pro
 685 690 695

10 CCA CAG ATC ATG TGG TTT AAA GAT AAT GAG ACC CTT 2121
 Pro Gln Ile Met Trp Phe Lys Asp Asn Glu Thr Leu
 700 705

15 GTA GAA GAC TCA GGC ATT GTA TTG AAG GAT GGG AAC 2157
 Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn
 710 715

20 CGG AAC CTC ACT ATC CGC AGA GTG AGG AAG GAG GAC 2193
 Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp
 25 720 725 730

30 GAA GGC CTC TAC ACC TGC CAG GCA TGC AGT GTT CTT 2229
 Glu Gly Leu Tyr Thr Cys Gln Ala Cys Ser Val Leu
 735 740

35 GGC TGT GCA AAA GTG GAG GCA TTT TTC ATA ATA GAA 2265
 Gly Cys Ala Lys Val Glu Ala Phe Phe Ile Ile Glu
 745 750 755

40 GGT GCC CAG GAA AAG ACG AAC TTG GAA ATC ATT ATT 2301
 Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile
 760 765

45 CTA GTA GGC ACG ACG GTG ATT GCC ATG TTC TTC TGG 2337
 Leu Val Gly Thr Thr Val Ile Ala Met Phe Phe Trp
 770 775

50 CTA CTT CTT GTC ATC ATC CTA GGG ACC GTT AAG CGG 2373
 Leu Leu Leu Val Ile Ile Leu Gly Thr Val Lys Arg
 55 780 785 790

5 GCC AAT GGA GGG GAA CTG AAG ACA GGC TAC TTG TCC 2409
 Ala Asn Gly Gly Glu Leu Lys Thr Gly Tyr Leu Ser
 795 800

10 ATC GTC ATG GAT CCA GAT GAA CTC CCA TTG GAT GAA 2445
 Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu
 805 810 815

15 CAT TGT GAA CGA CTG CCT TAT GAT GCC AGC AAA TGG 2481
 His Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys Trp
 820 825

20 GAA TTC CCC AGA GAC CGG CTG AAC CTA GGT AAG CCT 2517
 Glu Phe Pro Arg Asp Arg Leu Asn Leu Gly Lys Pro
 830 835

25 CTT GGC CGT GGT GCC TTT GGC CAA GAG ATT GAA GCA 2553
 Leu Gly Arg Gly Ala Phe Gly Gln Glu Ile Glu Ala
 840 845 850

30 GAT GCC TTT GGA ATT GAC AAG ACA GCA ACT TGC AGG 2589
 Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Arg
 855 860

35 ACA GTA GCA GTC AAA ATG TTG AAA GAA GGA GCA ACA 2625
 Thr Val Ala Val Lys Met Leu Lys Glu Gly Ala Thr
 865 870 875

40 CAC AGT GAG CAT CGA GCT CTC ATG TCT GAA CTC AAG 2661
 His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys
 880 885

45 ATC CTC ATT CAT ATT GGT CAC CAT CTC AAT GTG GTC 2697
 Ile Leu Ile His Ile Gly His His Leu Asn Val Val
 890 895

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5 AAC CTT CTA GGT GCC TGT ACC AAG CCA GGA GGG CCA 2733
 Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro
 900 905 910

10 CTC ATG GTG ATT GTG GAA TTC TGC AAA TTT GGA AAC 2769
 Leu Met Val Ile Val Glu Phe Cys Lys Phe Gly Asn
 915 920

15 CTG TCC ACT TAC CTG AGG AGC AAG AGA AAT GAA TTT 2805
 Leu Ser Thr Tyr Leu Arg Ser Lys Arg Asn Glu Phe
 925 930 935

20 GTC CCC TAC AAG ACC AAA GGG GCA CGA TTC CGT CAA 2841
 Val Pro Tyr Lys Thr Lys Gly Ala Arg Phe Arg Gln
 940 945

25 GGG AAA GAC TAC GTT GGA GCA ATC CCT GTG GAT CTG 2877
 Gly Lys Asp Tyr Val Gly Ala Ile Pro Val Asp Leu
 950 955

30 AAA CGG CGC TTG GAC AGC ATC ACC AGT AGC CAG AGC 2913
 Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser
 960 965 970

35 TCA GCC AGC TCT GGA TTT GTG GAG GAG AAG TCC CTC 2949
 Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu
 975 980

40 AGT GAT GTA GAA GAA GAG GAA GCT CCT GAA GAT CTG 2985
 Ser Asp Val Glu Glu Glu Glu Ala Pro Glu Asp Leu
 985 990 995

45 TAT AAG GAC TTC CTG ACC TTG GAG CAT CTC ATC TGT 3021
 Tyr Lys Asp Phe Leu Thr Leu Glu His Leu Ile Cys
 1000 1005

55

5 TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC TTG 3057
 Tyr Ser Phe Gln Val Ala Lys Gly Met Glu Phe Leu
 1010 1015

10 GCA TCG CGA AAG TGT ATC CAC AGG GAC CTG GCG GCA 3093
 Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala
 1020 1025 1030

15 CGA AAT ATC CTC TTA TCG GAG AAG AAC GTG GTT AAA 3129
 Arg Asn Ile Leu Leu Ser Glu Lys Asn Val Val Lys
 1035 1040

20 ATC TGT GAC TTT GGC TTG GCC CGG GAT ATT TAT AAA 3165
 Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys
 25 1045 1050 1055

30 GAT CCA GAT TAT GTC AGA AAA GGA GAT GCT CGC CTC 3201
 Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg Leu
 1060 1065

35 CCT TTG AAA TGG ATG GCC CCA GAA ACA ATT TTT GAC 3237
 Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp
 1070 1075

40 AGA GTG TAC ACA ATC CAG AGT GAC GTC TGG TCT TTT 3273
 Arg Val Tyr Thr Ile Gln Ser Asp Val Trp Ser Phe
 1080 1085 1090

45 GGT GTT TTG CTG TGG GAA ATA TTT TCC TTA GGT GCT 3309
 Gly Val Leu Leu Trp Glu Ile Phe Ser Leu Gly Ala
 1095 1100

50 TCT CCA TAT CCT GGG GTA AAG ATT GAT GAA GAA TTT 3345
 Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu Phe
 55 1105 1110 1115

5 TGT AGG CGA TTG AAA GAA GGA ACT AGA ATG AGG GCC 3381
 Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala
 1120 1125

10 CCT GAT TAT ACT ACA CCA GAA ATG TAC CAG ACC ATG 3417
 Pro Asp Tyr Thr Thr Pro Glu Met Tyr Gln Thr Met
 1130 1135

15 CTG GAC TGC TGG CAC GGG GAG CCC AGT CAG AGA CCC 3453
 Leu Asp Cys Trp His Gly Glu Pro Ser Gln Arg Pro
 1140 1145 1150

20 ACG TTT TCA GAG TTG GTG GAA CAT TTG GGA AAT CTC 3489
 Thr Phe Ser Glu Leu Val Glu His Leu Gly Asn Leu
 25 1155 1160

30 TTG CAA GCT AAT GCT CAG CAG GAT GGC AAA GAC TAC 3525
 Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr
 1165 1170 1175

35 ATT GTT CTT CCG ATA TCA GAG ACT TTG AGC ATG GAA 3561
 Ile Val Leu Pro Ile Ser Glu Thr Leu Ser Met Glu
 1180 1185

40 GAG GAT TCT GGA CTC TCT CTG CCT ACC TCA CCT GTT 3597
 Glu Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro Val
 1190 1195

45 TCC TGT ATG GAG GAG GAG GAA GTA TGT GAC CCC AAA 3633
 Ser Cys Met Glu Glu Glu Glu Val Cys Asp Pro Lys
 50 1200 1205 1210

55 TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAG TAT 3669
 Phe His Tyr Asp Asn Thr Ala Gly Ile Ser Gln Tyr
 1215 1220

5 CTG CAG AAC AGT AAG CGA AAG AGC CGG CCT GTG AGT 3705
 Leu Gln Asn Ser Lys Arg Lys Ser Arg Pro Val Ser
 1225 1230 1235

10 GTA AAA ACA TTT GAA GAT ATC CCG TTA GAA GAA CCA 3741
 Val Lys Thr Phe Glu Asp Ile Pro Leu Glu Glu Pro
 1240 1245

15 GAA GTA AAA GTA ATC CCA GAT GAC AAC CAG ACG GAC 3777
 Glu Val Lys Val Ile Pro Asp Asp Asn Gln Thr Asp
 1250 1255

20 AGT GGT ATG GTT CTT GCC TCA GAA GAG CTG AAA ACT 3813
 Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr
 1260 1265 1270

25 TTG GAA GAC AGA ACC AAA TTA TCT CCA TCT TTT GGT 3849
 Leu Glu Asp Arg Thr Lys Leu Ser Pro Ser Phe Gly
 1275 1280

30 GGA ATG GTG CCC AGC AAA AGC AGG GAG TCT GTG GCA 3885
 Gly Met Val Pro Ser Lys Ser Arg Glu Ser Val Ala
 1285 1290 1295

35 TCT GAA GGC TCA AAC CAG ACA AGC GGC TAC CAG TCC 3921
 Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln Ser
 1300 1305

40 GGA TAT CAC TCC GAT GAC ACA GAC ACC ACC GTG TAC 3957
 Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr
 1310 1315

45 TCC AGT GAG GAA GCA GAA CTT TTA AAG CTG ATA GAG 3993
 Ser Ser Glu Glu Ala Glu Leu Leu Lys Leu Ile Glu
 1320 1325 1330

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5 ATT GGA GTG CAA ACC GGT AGC ACA GCC CAG ATT CTC 4029
 Ile Gly Val Gln Thr Gly Ser Thr Ala Gln Ile Leu
 1335 1340

10 CAG CCT GAC ACG GGG ACC ACA CTG AGC TCT CCT CCT 4065
 Gln Pro Asp Thr Gly Thr Thr Leu Ser Ser Pro Pro
 1345 1350 1355

15 GTT TAAAAGGAAG CATCCACACC CCAACTCCCG GACATCACAT 4108
 Val
 1356

20 GAGAGGTCTG CTCAGATTTT GAAGTGTGT TCTTTCCACC 4148

25 AGCAGGAAGT AGCCGCATTT GATTTTCATT TCGACAACAG 4188

 AAAAAGGACC TCGGACTGCA GGGAGCCAGC TCTTCTAGGC 4228

30 TTGTGACC 4236

(2) INFORMATION FOR SEQ ID NO: 8:

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(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 433 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

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- (A) NAME/KEY: ckit proto-oncogene receptor
- (B) LOCATION: Amino acids 543-975

(x) PUBLICATION INFORMATION:

50

- (A) AUTHORS: Yarden, Y., et al.
- (B) JOURNAL: EMBO J.
- (C) VOLUME: 6
- (D) PAGES: 3341-3351
- (E) DATE: 1987

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

5 Leu Thr Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln
 543 545 550 555

 10 Trp Lys Val Val Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr
 560 565 570

 15 Ile Asp Pro Thr Gln Leu Pro Tyr Asp His Lys Trp Glu Phe
 575 580

 20 Pro Arg Asn Arg Leu Ser Phe Gly Lys Thr Leu Gly Ala Gly
 585 590 595

 25 Ala Phe Gly Lys Val Val Ala Glu Thr Ala Tyr Gly Leu Ile
 600 605 610

 30 Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met Leu Lys

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	615	620	625
5	Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu		
	630	635	640
10	Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val		
	645	650	
15	Asn Leu Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val		
	655	660	665
20	Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu		
	670	675	680
25	Arg Arg Lys Arg Asp Ser Phe Ile Cys Ser Lys Gln Glu Asp		
	685	690	695
30	His Ala Glu Ala Ala Leu Tyr Lys Asn Leu Leu His Ser Lys		
	700	705	710
35	Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu Tyr Met Asp Met		
	715	720	
40	Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala Asp Lys		
	725	730	735
45	Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val		
	740	745	750
50	Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu		
	755	760	765
55	Glu Asp Leu Leu Ser Phe Ser Tyr Gln Val Lys Gly Met Ala		
	770	775	780

Phe Leu Ala Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala
 785 790
 5
 Arg Asn Ile Leu Leu Thr His Gly Arg Ile Thr Lys Ile Cys
 795 800 805
 10
 Asp Phe Gly Leu Ala Arg Asp Ile Lys Asn Asp Ser Asn Tyr
 810 815 820
 15
 Val Val Lys Gly Asn Ala Arg Leu Pro Val Lys Val Met Ala
 825 830 835
 20
 Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Glu Glu Ser Asp
 840 845 850
 25
 Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser Leu
 855 860
 30
 Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Lys Ser Lys Phe
 865 870 875
 35
 Tyr Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu
 880 885 890
 40
 His Ala Pro Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp
 895 900 905
 45
 Asp Ala Asp Pro Leu Lys Arg Pro Thr Phe Lys Gln Ile Val
 910 915 920
 50
 Gln Leu Ile Glu Lys Gln Ile Ser Glu Ser Thr Asn His Ile
 925 930
 55
 Tyr Ser Asn Leu Ala Asn Cys Ser Pro Asn Arg Gln Lys Pro
 935 940 945

Val Val Asp His Ser Val Arg Ile Asn Ser Val Gly Ser Thr
 5 950 955 960

Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
 10 965 970 975

(2) INFORMATION FOR SEQ ID NO: 9:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 437 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

25 (A) NAME/KEY: CSF-1 receptor

(B) LOCATION: Amino acids 536-972

(x) PUBLICATION INFORMATION:

30 (A) AUTHORS: Coussens, L., et al.

(B) JOURNAL: Nature

(C) VOLUME: 320

(D) PAGES: 277-280

35 (E) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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5 Leu Leu Tyr Lys Tyr Lys Gln Lys Pro Lys Tyr Gln Val Arg
 536 540 545

10 Trp Lys Ile Ile Glu Ser Tyr Glu Gly Asn Ser Tyr Thr Phe
 550 555 560

15 Ile Asp Pro Thr Gln Leu Pro Tyr Asn Glu Lys Trp Glu Phe
 565 570 575

20 Pro Arg Asn Asn Leu Gln Phe Gly Lys Thr Leu Gly Ala Gly
 580 585 590

25 Ala Phe Gly Lys Val Val Glu Ala Thr Ala Phe Gly Leu Gly
 595 600 605

30 Lys Glu Asp Ala Val Leu Lys Val Ala Val Lys Met Leu Lys
 610 615

35 Ser Thr Ala His Ala Asp Glu Lys Glu Ala Leu Met Ser Glu
 620 625 630

40 Leu Lys Ile Met Ser His Leu Gly Gln His Glu Asn Ile Val
 635 640 645

45 Asn Leu Leu Gly Ala Cys Thr His Gly Gly Pro Val Leu Val
 650 655 660

50 Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu
 665 670 675

55

Arg Arg Lys Ala Glu Ala Met Leu Gly Pro Ser Leu Ser Pro
680 685

Gly Gln Asp Pro Glu Gly Gly Val Asp Tyr Lys Asn Ile His
690 695 700

Leu Glu Lys Lys Tyr Val Arg Arg Asp Ser Gly Phe Ser Ser
705 710 715

Gln Gly Val Asp Thr Tyr Val Glu Met Arg Pro Val Ser Thr
720 725 730

Ser Ser Asn Asp Ser Phe Ser Glu Gln Asp Leu Asp Lys Glu
735 740 745

Asp Gly Arg Pro Leu Glu Leu Arg Asp Leu Leu His Phe Ser
750 755

Ser Gln Val Ala Gln Gly Met Ala Phe Leu Ala Ser Lys Asn
760 765 770

Cys Ile His Arg Asp Val Ala Ala Arg Asn Val Leu Leu Thr
775 780 785

Asn Gly His Val Ala Lys Ile Gly Asp Phe Gly Leu Ala Arg
790 795 800

Asp Ile Met Asn Asp Ser Asn Tyr Ile Val Lys Gly Asn Ala
805 810 815

Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asp
820 825

Cys Val Tyr Thr Val Gln Ser Asp Val Trp Ser Tyr Gly Ile
830 835 840

5 Leu Leu Trp Glu Ile Phe Ser Leu Gly Leu Asn Pro Tyr Pro
 845 850 855

 10 Gly Ile Leu Val Asn Ser Lys Phe Tyr Lys Leu Val Lys Asp
 860 865 870

 15 Gly Tyr Gln Met Ala Gln Pro Ala Phe Ala Pro Lys Asn Ile
 875 880 885

 20 Tyr Ser Ile Met Gln Ala Cys Trp Ala Leu Glu Pro Thr His
 890 895

 25 Arg Pro Thr Phe Gln Gln Ile Cys Ser Phe Leu Gln Glu Gln
 900 905 910

 30 Ala Gln Glu Asp Arg Arg Glu Arg Asp Tyr Thr Asn Leu Pro
 915 920 925

 35 Ser Ser Ser Arg Ser Gly Gly Ser Gly Ser Ser Ser Ser Glu
 930 935 940

 40 Leu Glu Glu Glu Ser Ser Ser Glu His Leu Thr Cys Cys Glu
 945 950 955

 45 Gln Gly Asp Ile Ala Gln Pro Leu Leu Gln Pro Asn Asn Tyr
 960 965

 50 Gln Phe Cys
 970

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 566 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: PDGF receptor
(B) LOCATION: Amino acids 522-1087

(x) PUBLICATION INFORMATION:

5

(A) AUTHORS: Gronwald, R., et al.
(B) JOURNAL: Proc. Natl. Acad. Sci.
(C) VOLUME: 85
(D) PAGES: 3435-3439
(E) DATE: 1988

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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Met Leu Trp Gln Lys Lys Pro Arg Tyr Glu Ile Arg Trp Lys
522 525 530 535

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Val Ile Glu Ser Val Ser Ser Asp Gly His Glu Tyr Ile Tyr
 540 545

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Val Asp Pro Val Gln Leu Pro Tyr Asp Ser Thr Trp Glu Leu
550 555 560

10

Pro Arg Asp Gln Leu Val Leu Gly Arg Thr Leu Gly Ser Gly
565 570 575

15

Ala Phe Gly Gln Val Val Glu Ala Thr Ala His Gly Leu Ser
580 585 590

20

His Ser Gln Ala Thr Met Lys Val Ala Val Lys Met Leu Lys
595 600 605

25

Ser Thr Ala Arg Ser Ser Glu Lys Gln Ser Leu Met Ser Glu
610 615

30

Leu Lys Ile Met Ser His Leu Gly Pro His Leu Asn Val Val
620 625 630

35

Asn Leu Leu Gly Ala Cys Thr Lys Gly Gly Pro Ile Tyr Ile
635 640 645

40

Ile Thr Glu Tyr Cys Arg Tyr Gly Asp Leu Val Asp Tyr Leu
650 655 660

45

His Arg Asn Lys His Thr Phe Leu Gln Arg His Ser Asn Lys
665 670 675

50

His Cys Pro Pro Ser Ala Glu Leu Tyr Ser Asn Ala Leu Pro
680 685

55

Val Gly Phe Ser Leu Pro Ser His Leu Asn Leu Thr Gly Glu
690 695 700

Ser Asp Gly Gly Tyr Met Asp Met Ser Lys Asp Glu Ser Ile
705 710 715

Asp Tyr Val Pro Met Leu Asp Met Lys Gly Asp Ile Lys Tyr
 720 725 730
 5

Ala Asp Ile Glu Ser Pro Ser Tyr Met Ala Pro Tyr Asp Asn
 735 740 745
 10

Tyr Val Pro Ser Ala Pro Glu Arg Thr Tyr Arg Ala Thr Leu
 750 755
 15

Ile Asn Asp Ser Pro Val Leu Ser Tyr Thr Asp Leu Val Gly
 760 765 770
 20

Phe Ser Tyr Gln Val Ala Asn Gly Met Asp Phe Leu Ala Ser
 775 780 785
 25

Lys Asn Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu
 790 795 800
 30

Ile Cys Glu Gly Lys Leu Val Lys Ile Cys Asp Phe Gly Phe
 805 810 815
 35

Ala Arg Asp Ile Met Arg Asp Ser Asn Tyr Ile Ser Lys Gly
 820 825
 40

Ser Thr Tyr Leu Pro Leu Lys Trp Met Ala Pro Glu Ser Ile
 830 835 840
 45

Phe Asn Ser Leu Tyr Thr Thr Leu Ser Asp Val Trp Ser Phe
 845 850 855
 50

Gly Ile Leu Leu Trp Glu Ile Phe Thr Leu Gly Gly Thr Pro
 860 865 870
 55

Tyr Pro Glu Leu Pro Met Asn Asp Gln Phe Tyr Asn Ala Ile
 875 880 885

Lys Arg Gly Tyr Arg Met Ala Gln Pro Ala His Ala Ser Asp
 890 895
 5
 Glu Ile Tyr Glu Ile Met Gln Lys Cys Trp Glu Glu Lys Phe
 900 905 910
 10
 Glu Thr Arg Pro Pro Phe Ser Gln Leu Val Leu Leu Leu Glu
 915 920 925
 15
 Arg Leu Leu Gly Glu Gly Tyr Lys Lys Lys Tyr Gln Gln Val
 930 935 940
 20
 Asp Glu Glu Phe Leu Arg Ser Asp His Pro Ala Ile Leu Arg
 945 950 955
 25
 Ser Gln Ala Arg Phe Pro Gly Ile His Ser Leu Arg Ser Pro
 960 965
 30
 Leu Asp Thr Ser Ser Val Leu Tyr Thr Ala Val Gln Pro Asn
 970 975 980
 35
 Glu Ser Asp Asn Asp Tyr Ile Ile Pro Leu Pro Asp Pro Lys
 985 990 995
 40
 Pro Asp Val Ala Asp Glu Gly Leu Pro Glu Gly Ser Pro Ser
 1000 1005 1010
 45
 Leu Ala Ser Ser Thr Leu Asn Glu Val Asn Thr Ser Ser Thr
 1015 1020 1025
 50
 Ile Ser Cys Asp Ser Pro Leu Glu Leu Gln Glu Glu Pro Gln
 1030 1035
 55
 Gln Ala Glu Pro Glu Ala Gln Leu Glu Gln Pro Gln Asp Ser
 1040 1045 1050

Gly Cys Pro Gly Pro Leu Ala Glu Ala Glu Asp Ser Phe Leu
1055 1060 1065

Glu Gln Pro Gln Asp Ser Gly Cys Pro Gly Pro Leu Ala Glu
1070 1075 1080

Ala Glu Asp Ser Phe Leu
1085

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TCGACGCGCG ATG GAG

16

Claims

1. A recombinant human DNA sequence encoding a human type III receptor tyrosine kinase, said DNA comprising the nucleotide sequence of the inserts of clones BTIII081.8 (ATCC accession number 40,931) and BTIII129.5 (ATCC accession number 40,975) or a corresponding nucleotide sequence by virtue of the redundancy of the genetic code.
2. A 363 base pair nucleic acid having the sequence of SEQ ID NO: 3
3. An expression vector comprising the recombinant human DNA sequence of claim 1.
4. A lambda gt11 phage harboring the clone BTIII081.8 (ATCC accession number 40,931) or the clone BTIII129.5 (ATCC accession number 40,975).
5. A protein encoded by the sequence of claim 1.
6. An oligonucleotide primer consisting of 27 bases and having the sequence of SEQ ID NO: 1.
7. An oligonucleotide primer consisting of 35 bases and having the sequence of SEQ ID NO: 2.
8. A method for the expression of a protein defined by claim 5 which comprises transforming a host cell with the expression vector of claim 3 or 4 and culturing the transformed host cell under conditions which result in expression of the protein by the expression vector.

9. The method of claim 8, wherein the host cell is a bacteria, virus, yeast, insect or mammalian cell line.
10. The method of claim 9 wherein the host cell is a COS-1 cell, NIH3T3 fibroblast or CMT-3 monkey kidney cell.
11. Use of a biologically active human type III receptor tyrosine kinase comprising the sequence of the protein according to claim 5 in a screening of pharmaceuticals for antagonist or agonist vascular endothelial cell growth factor (VEGF) action on the human type III receptor tyrosine kinase.
12. Use according to claim 11 wherein the screening is a method comprising the steps of:
 - (a) incubating cells expressing the human type III receptor tyrosine kinase with [¹²⁵I] VEGF and a compound;
 - (b) measuring the emitted radioactive to determine the amount of inhibition of binding of VEGF to the human type III receptor tyrosine kinase by the compound.

Patentansprüche

1. Rekombinante humane DNA Sequenz, die für eine humane Typ-III Rezeptor-Tyrosin-Kinase kodiert, wobei die DNA Sequenz die Nukleotidsequenz der Inserts der Klone BTIII081.8 (ATCC Zugriffsnummer 40,931) und BTIII129.5 (ATCC Zugriffsnummer 40,975) oder eine entsprechende Nukleotidsequenz gemäß der Redundanz des genetischen Codes umfasst.
2. Nukleinsäure von 363 Basenpaaren mit der Sequenz von SEQ ID No:3.
3. Expressionsvektor, der die rekombinante humane DNA Sequenz von Anspruch 1 umfasst.
4. Lambda gt11 Phage, der den Klon BTIII081.8 (ATCC Zugriffsnummer 40,931) oder den Klon BTIII129.5 (ATCC Zugriffsnummer 40,975) enthält.
5. Protein, das von der Sequenz von Anspruch 1 kodiert wird.
6. Oligonukleotid-Primer bestehend aus 27 Basen der Sequenz von SEQ ID No: 1.
7. Oligonukleotid-Primer bestehend aus 35 Basen der Sequenz von SEQ ID No: 2.
8. Verfahren zur Expression eines Proteins wie in Anspruch 5 definiert, umfassend das Transformieren einer Wirtszelle mit dem Expressionsvektor von Anspruch 3 oder 4 und Kultivieren der transformierten Wirtszelle unter Bedingungen, die zur Expression des Proteins durch den Expressionsvektor führen.
9. Verfahren nach Anspruch 8, wobei die Wirtszelle zur Zelllinie eines Bakterium, eines Virus, einer Hefe, eines Insekts oder eines Säugers gehört.
10. Verfahren nach Anspruch 9, wobei die Wirtszelle eine COS-1 Zelle, ein NIH3T3 Fibroblast oder eine CMT-3 Nierenzelle eines Affen ist.
11. Verwendung einer biologisch aktiven humanen Typ-III Rezeptor-Tyrosin-Kinase, die die Sequenz des Proteins gemäß Anspruch 5 umfasst, in einem pharmazeutischen Screening nach Antagonisten oder Agonisten der Wirkung des Gefäß-Endothelzellen-Wachstumsfaktors (vascular endothelial cell growth factor, VEGF) auf die humane Typ-III Rezeptor-Tyrosin-Kinase.
12. Verwendung nach Anspruch 11, wobei das Screening ein Verfahren umfassend die folgenden Schritte ist:
 - (a) Inkubation von Zellen, die die humane Typ-III Rezeptor-Tyrosin-Kinase exprimieren, mit [¹²⁵I]-VEGF und einer Verbindung,
 - (b) Messen der emittierten Radioaktivität zur Bestimmung des Ausmaßes der Inhibition der Bindung von VEGF an die humane Typ-III Rezeptor-Tyrosin-Kinase durch die Verbindung.

Revendications

- 5 1. Séquence d'ADN humain recombinant codant pour le récepteur de la tyrosine kinase humain de type III, ledit ADN comprenant la séquence nucléotidique des inserts des clones BTIII081.8 (déposé auprès de l'ATCC sous le numéro 40 931) et BTIII129.5 (déposé auprès de l'ATCC sous le numéro 40 975) ou une séquence nucléotidique correspondante en vertu de la redondance du code génétique.
2. Acide nucléique de 363 paires de bases possédant la séquence de SEQ ID N°3.
- 10 3. Vecteur d'expression comprenant la séquence d'ADN humain recombinant de la revendication 1.
4. Phage lambda gt11 contenant le clone BT111081.8 (déposé auprès de l'ATCC sous le numéro 40 931) ou le clone BTIII129.5 (déposé auprès de l'ATCC sous le numéro 40 975).
- 15 5. Protéine codée par la séquence de la revendication 1.
6. Amorce oligonucléotidique constituée de 27 bases et possédant la séquence de SEQ ID N°1.
7. Amorce oligonucléotidique constituée de 35 bases et possédant la séquence de SEQ ID N°2.
- 20 8. Procédé pour l'expression d'une protéine définie à la revendication 5 qui comprend la transformation d'une cellule hôte avec le vecteur d'expression de la revendication 3 ou 4 et la culture de la cellule hôte transformée dans des conditions qui aboutissent à l'expression de la protéine par le vecteur d'expression.
- 25 9. Procédé de la revendication 8, dans lequel la cellule hôte est une bactérie, un virus, une levure, ou une lignée cellulaire d'insecte ou de mammifère.
10. Procédé selon la revendication 9 dans lequel la cellule hôte est une cellule COS-1, un fibroblaste NIH3T3 ou une cellule de rein de singe CMT-3.
- 30 11. Utilisation d'un récepteur de la tyrosine kinase humain de type III comprenant la séquence de la protéine selon la revendication 5 pour cribler des produits pharmaceutiques pour leur activité antagoniste ou agoniste du facteur de croissance des cellules endothéliales vasculaires sur le récepteur de la tyrosine kinase humain de type III.
- 35 12. Utilisation selon la revendication 11 où le procédé de criblage comprend les étapes consistant à :
 - (a) incuber des cellules exprimant le récepteur de la tyrosine kinase humain de type III avec le VEGF marqué à 1' [¹²⁵I] et un composé ; et à
 - 40 (b) mesurer la radioactivité émise afin de déterminer la quantité d'inhibition de la liaison du VEGF au récepteur de la tyrosine kinase humain de type III par le composé.

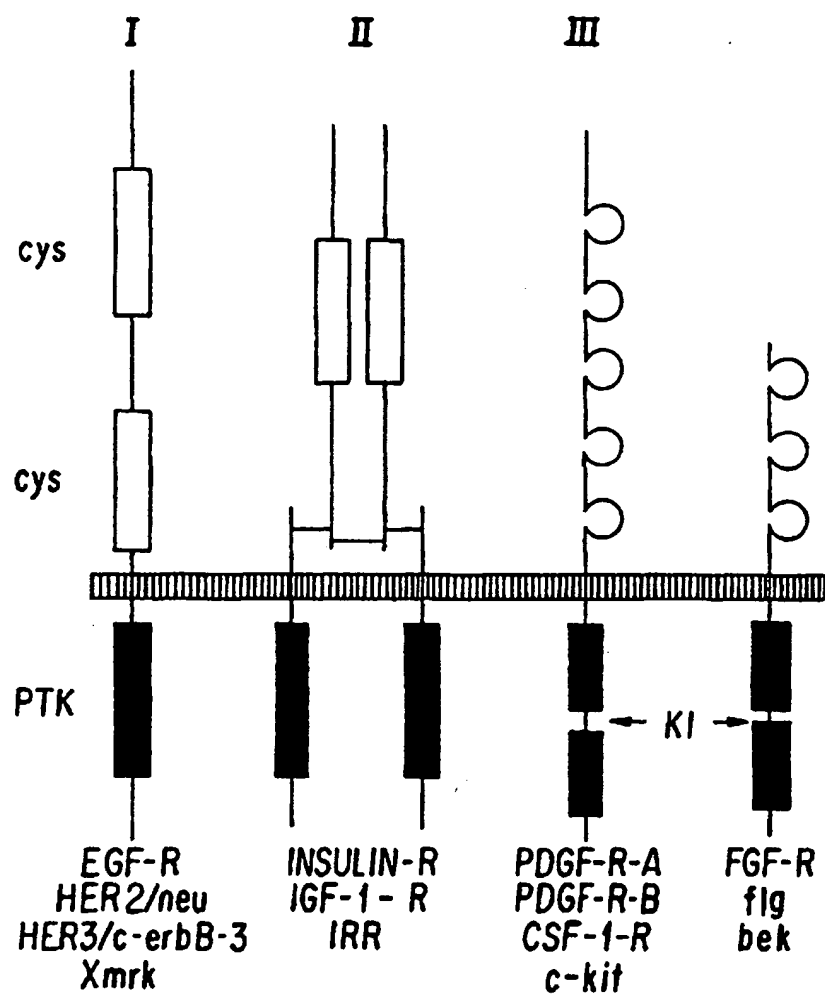


FIG. 1

PRIMER 1

RECEPTOR

PDGF	AAC CTG TTG GGG GCC TGC ACC
ckit	T A T A
CSF	T A
FGF	C G

PRIMER 1

GTG GAC AAC CTG TTG GGG GCC TGC AAC
T A

PRIMER 2

RECEPTOR

PDGF	CAC AGA GAC CTG GCG GCT AGG AAC GTG CT
ckit	T GA C A T A
CSF	C G G A GC C T
FGF	C C C T C

CONSENSUS

CAC AGA GAC CTG GCG GCT AGI AAC GTG CT
C T C T

PRIMER 2

GAATTG AG CAC GTT ICT AGC CGC CAG GTC TCT GTG
T G T G

FIG.2

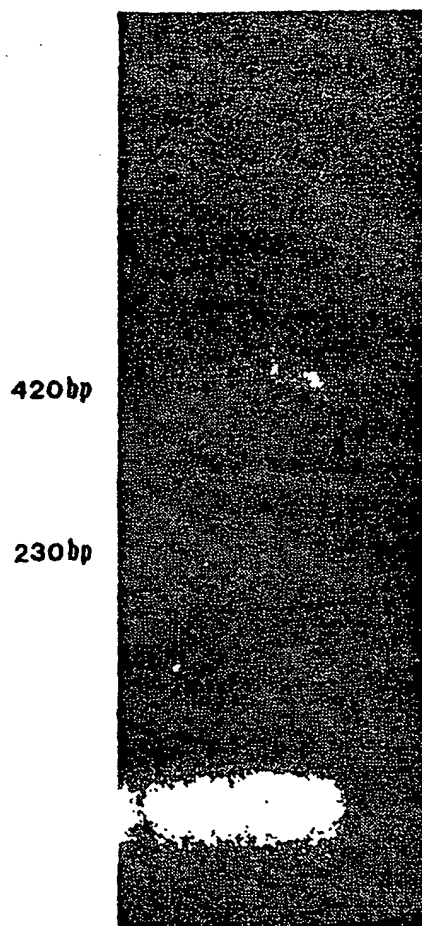


FIG. 3

FIG. 4A

FIG. 4B

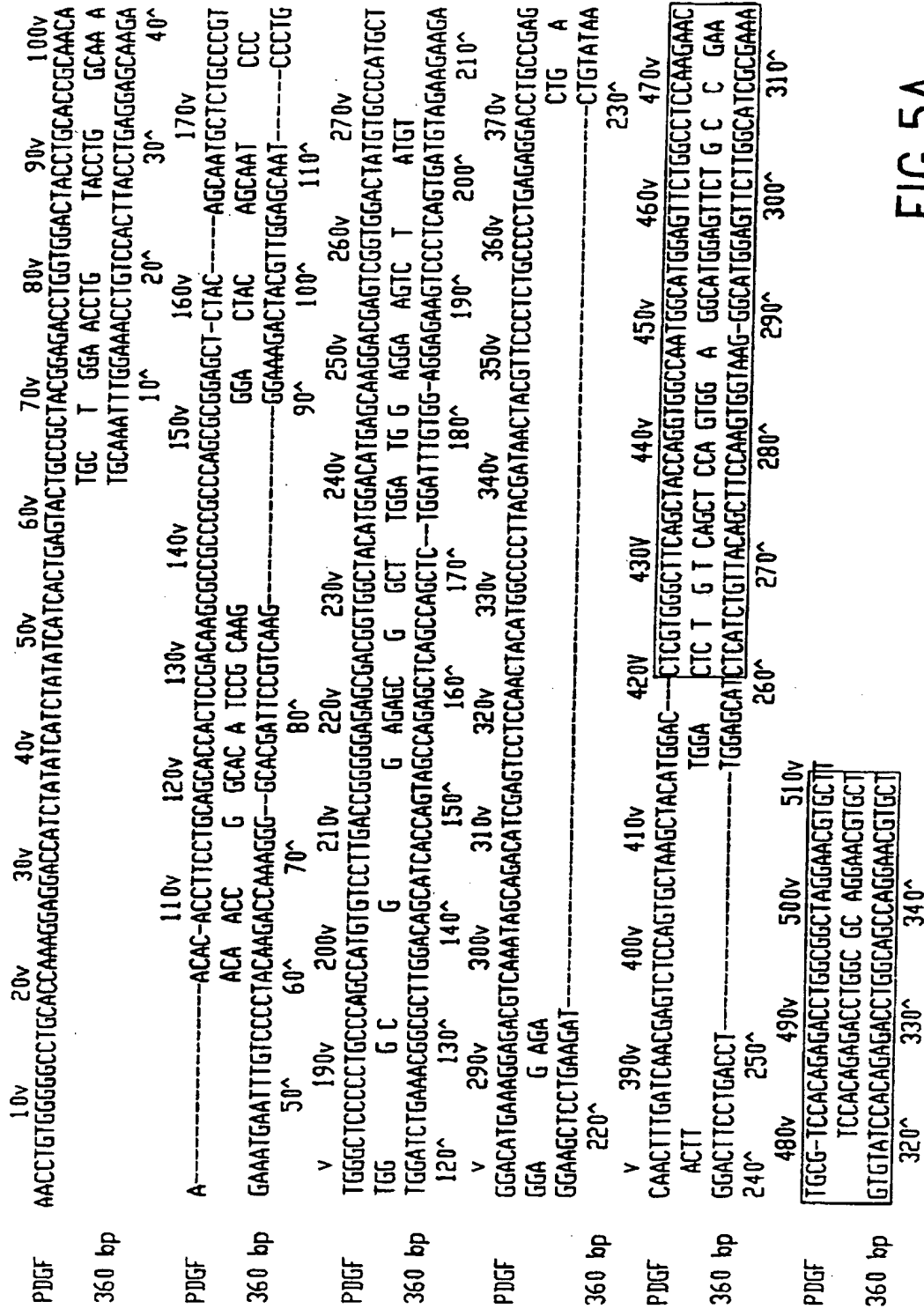


FIG.5A

	10v	20v	30v	40v	50v	60v	70v	80v	
FGF	AACCTGCTGGGGGCTGCACGAGGATGGTCCCTTGATGTCATGCTGGAGTATGCCCTCCAAAGGGCAACCTGCGGGAGTACCTGC-----								
	AA	CTG	TGGGGGCTGCAC	CCT	CGT	CTC	A	CAAC	A ACC G
230 bp	AATCTGTTGGGGGCTGCACCATCCCAACATCCTG-----CGT-----CTCTA-----CAACTATTTTATGACCGGAGGAGGATCTACTTGAT								
	10^	20^	30^	40^	50^	60^	70^		
	90v	100v	110v	120v	130v	140v	150v	160v	170v 180v
FGF1	---AGACCCGGAGGCCCCCAGGGCTGGAACTACTGCTATAACCCAGCCACACCCAGAGGAGCAGCTCTCTCTCCAAAGGACCTGGTGTCTCTGGCGCTACCA								
	AGA	G	CCC	C	G	GCT	A	A	AGC CA GA GAGCAGC C CCA G C TGG A
230 bp	TCTAGAGTATGCCCCCGGGGAGCTCTACAAGGAGCTGCAGAGAGCTGCACATTTGACGAGCAGCGAACAGCCACCGATCATGG-----A								
	90^	100^	110^	120^	130^	140^	150^	160^	
	190v	200v	210v	220v	230v	240v	250v		
FGF	GGA----GGCCCGAGGCGATGGAGTATCTGGCTCCAAAGAGTGCTACACCTGAGACCTGGCAGCCAGGAATGTCTG								
	GGA	GGC	G	T	GTA	AAGAAG	AT	CAC	GAGACCTGGCAGCCAG AA GT CT
230 bp	GGAGTTGGCAGATGCTCTAATGTACTGCCCTGGGAAGAGGTGATTACACAGAGACCTGGCAGCCAGCAACGTGCT								
	170^	180^	190^	200^	210^	220^	230^		

FIG.5B

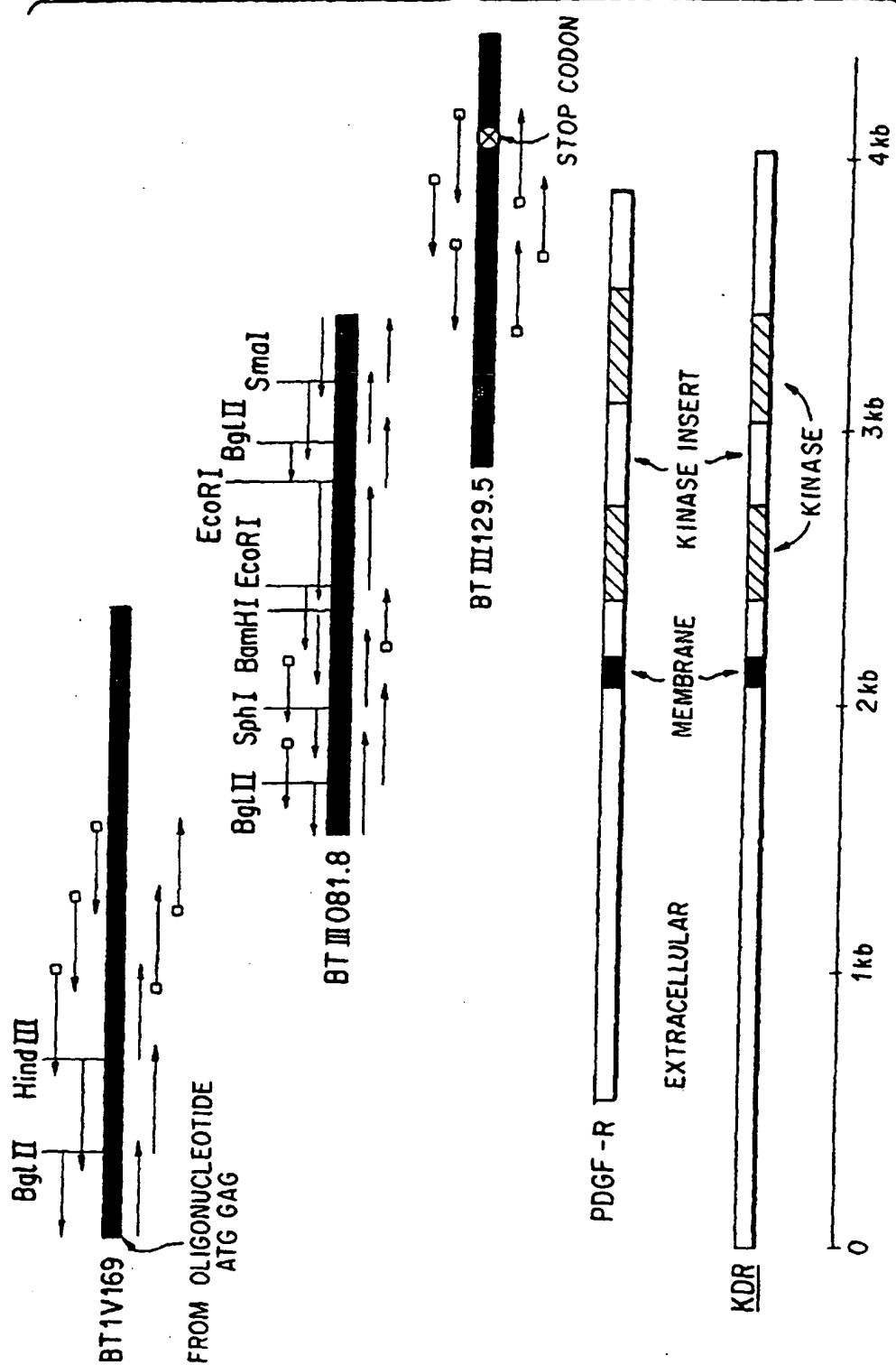


FIG. 6

10	20	30	40	50
ATG GAG AGC AAG GTG CTG CTG GCC GTC GCC CTG TGG CTC TGC GTG GAG ACC CGG				*
Met Glu Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu			Cys	Val Glu Thr Arg>
60	70	80	90	100
GCC GCC TCT GTG GGT TTG CCT AGT GGT TCT CTT GAT CTG CCC AGG CTC AGC ATA				*
Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro Arg Leu Ser Ile>				
110	120	130	140	150
CAA AAA GAC ATA CTT ACA ATT AAG GCT AAT ACA ACT CTT CAA ATT ACT TGC AGG			*	*
Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr Leu Gln Ile Thr				Cys Arg>
170	180	190	200	210
GGA CAG AGG GAC TTG GAC TGG CTT TGG CCC AAT AAT CAG AGT GGC AGT GAG CAA			*	*
Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu Gln>				
220	230	240	250	260
AGG GTG GAG GTG ACT GAG TGC AGC GAT GGC CTC TTC TGT AAG ACA CTC ACA ATT			*	*
Arg Val Glu Val Thr Glu	Cys	Ser Asp Gly Leu Phe	Cys	Lys Thr Leu Thr Ile>
280	290	300	310	320
CCA AAA GTG ATC GGA AAT GAC ACT GGA GCC TAC AAG TGC TTC TAC CGG GAA ACT			*	*
Pro Lys Val Ile Gly Asn Asp Thr Gly Ala Tyr Lys			Cys	Phe Tyr Arg Glu Thr>

FIG. 7A

```

330 *      340 *      350 *      360 *      370 *
GAC TTG GCC TCG GTC ATT TAT GTC TAT GTT CAA GAT TAC AGA TCT CCA TTT ATT
Asp Leu Ala Ser Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile>

380 *      390 *      400 *      410 *      420 *      430 *
GCT TCT GTT AGT GAC CAA CAT GGA GTC GTG TAC ATT ACT GAG AAC AAA AAC AAA
Ala Ser Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys>

440 *      450 *      460 *      470 *      480 *
ACT GTG GTG ATT CCA TGT CTC GGG TCC ATT TCA AAT CTC AAC GTG TCA CTT TGT
Thr Val Val Ile Ile Pro [Cys] Leu Gly Ser Ile Ser Asn Leu Asn Val Ser Leu [Cys]>

490 *      500 *      510 *      520 *      530 *      540 *
GCA AGA TAC CCA GAA AAG AGA TTT GTT CCT GAT GGT AAC AGA ATT TCC TGG GAC
Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp>

550 *      560 *      570 *      580 *      590 *
AGC AAG AAG GGC TTT ACT ATT CCC AGC TAC ATG ATC AGC TAT GCT GGC ATG GTC
Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile Ser Tyr Ala Gly Met Val>

600 *      610 *      620 *      630 *      640 *
TTC TGT GAA GCA AAA ATT AAT GAT GAA AGT TAC CAG TCT ATT ATG TAC ATA GTT
Phe [Cys] Glu Ala Lys Ile Asn Asp Glu Ser Tyr Gln Ser Ile Met Tyr Ile Val>

```

FIG. 7B

650	660	670	680	690	700
* GTC GTT GTA GGG TAT AGG ATT TAT GAT GTG GTT CTG AGT CCG TCT CAT GGA ATT Val Val Val Gly Tyr Tyr Arg Ile Tyr Asp Val Val Leu Ser Pro Ser His Gly Ile>					
710	720	730	740	750	
* GAA CTA TCT GTT GGA GAA AAG CTT GTC TTA AAT TGT ACA GCA AGA ACT GAA CTA Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu>					
760	770	780	790	800	810
* AAT GTG GGG ATT GAC TTC AAC TGG GAA TAC CCT TCT TCT TCG AAG CAT CAG CAT AAG Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys>					
820	830	840	850	860	
* AAA CTT GTA AAC CGA GAC CTA AAA ACC CAG TCT GGG AGT GAG ATG AAG AAA TTT Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe>					
870	880	890	900	910	
* TTG AGC ACC TTA ACT ATA GAT GGT GTA ACC CGG AGT GAC CAA GGA TTG TAC ACC Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr>					
920	930	940	950	960	970
* TGT GCA GCA TCC AGT GGG CTG ATG ACC AAG AAG AAC AGC ACA TTT GTC AGG GTC Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg Val>					

FIG. 7C

980	990	1000	1010	1020
CAT GAA AAA CCT TTT GTT GCT TTT GGA AGT GGC ATG GAA TCT CTG GTG GAA GCC His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu Val Glu Ala>				
1030	1040	1050	1060	1070
ACG GTG GGG GAG CGT GTC AGA ATC CCT GCG AAG TAC CTT GGT TAC CCA CCC CCA Thr Val Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro>				
1090	1100	1110	1120	1130
GAA ATA AAA TGG TAT AAA AAT GGA ATA CCC CTT GAG TCC AAT CAC ACA ATT AAA Glu Ile Lys Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys>				
1140	1150	1160	1170	1180
GCG GGG CAT GTA CTG ACG ATT ATG GAA GTG AGT GAA AGA GAC ACA GGA AAT TAC Ala Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr>				
1190	1200	1210	1220	1230
ACT GTC ATC CTT ACC AAT CCC ATT TCA AAG GAG AAG CAG AGC CAT GTG GTC TCT Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser>				
1250	1260	1270	1280	1290
CTG GTT GTG TAT GTC CCA CCC CAG ATT GGT GAG AAA TCT CTA ATC TCT CCT GTG Leu Val Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val>				

FIG. 7D

1300 * 1310 * 1320 * 1330 * 1340 * 1350 *
 GAT TCC TAC CAG TAC GGC ACC ACT CAA ACG CTG ACA TGT ACG GTC TAT GCC ATT
 Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr [Cys] Thr Val Tyr Ala Ile>

 1360 * 1370 * 1380 * 1390 * 1400 *
 CCT CCC CCG CAT CAC ATC CAC TGG TAT TGG CAG TTG GAG GAA GAG TGC GCC AAC
 Pro Pro Pro His His Ile His Tyr Tyr Tip Gln Leu Glu Glu [Cys] Ala Asn>

 1410 * 1420 * 1430 * 1440 * 1450 *
 GAG CCC AGC CAA GCT GTC TCA GTG ACA AAC CCA TAC CCT TGT GAA GAA TGG AGA
 Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr Pro [Cys] Glu Glu Trp Arg>

 1460 * 1470 * 1480 * 1490 * 1500 * 1510 *
 AGT GTG GAG GAC TTC CAG GGA GGA AAT AAA ATT GAA GTT AAT AAA AAT CAA TTT
 Ser Val Glu Asp Phe Gln Gly Gln Gly Asn Lys Ile Glu Val Asn Lys Asn Gln Phe>

 1520 * 1530 * 1540 * 1550 * 1560 *
 GCT CTA ATT GAA GGA AAA AAC AAA ACT GTA AGT ACC CTT GTT ATC CAA GCG GCA
 Ala Leu Ile Glu Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala>

 1570 * 1580 * 1590 * 1600 * 1610 * 1620 *
 AAT GTG TCA GCT TTG TAC AAA TGT GAA GCG GTC AAC AAA GTC GGG AGA GGA GAG
 Asn Val Ser Ala Leu Tyr Lys [Cys] Glu Ala Val Asn Lys Val Gly Arg Gly Glu>

FIG. 7E

1630	1640	1650	1660	1670
*	*	*	*	*
AGG GTG ATC TCC TTC CAC GTG ACC AGG GGT CCT GAA ATT ACT TTG CAA CCT GAC				
Arg Val Ile Ser Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp>				
1680	1690	1700	1710	1720
*	*	*	*	*
ATG CAG CCC ACT GAG CAG GAG AGC GTG TCT TTG TGG TGC ACT GCA GAC AGA TCT				
Met Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Trp [Cys] Thr Ala Asp Arg Ser>				
1730	1740	1750	1760	1770
*	*	*	*	*
ACG TTT GAG AAC CTC ACA TGG TAC AAG CTT GGC CCA CAG CCT CTG CCA ATC CAT				
Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro Ile His>				
1790	1800	1810	1820	1830
*	*	*	*	*
GTG GGA GAG TTG CCC ACA CCT GTT TGC AAG AAC TTG GAT ACT CTT TGG AAA TTG				
Val Gly Glu Leu Pro Thr Pro Val [Cys] Lys Asn Leu Asp Thr Leu Trp Lys Leu>				
1840	1850	1860	1870	1880
*	*	*	*	*
AAT GCC ACC ATG TTC TCT AAT AGC ACA AAT GAC ATT TTG ATC ATG GAG CTT AAG				
Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile Met Glu Leu Lys>				
1900	1910	1920	1930	1940
*	*	*	*	*
AAT GCA TCC TTG CAG GAC CAA GGA GAC TAT GTC TGC CTT GCT CAA GAC AGG AAG				
Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr Val Cys Leu Ala Gln Asp Arg Lys>				

FIG. 7F

1950	1960	1970	1980	1990
ACC AAG AAA AGA CAT TGC GTG GTC AGG CAG CTC ACA GTC CTA GAG CGT GTG GCA Thr Lys Lys Arg His [Cys] Val Val Arg Gln Leu Thr Val Leu Glu Arg Val Ala>				
2000	2010	2020	2030	2040
CCC ACG ATC ACA GGA AAC CTG GAG AAT CAG ACG ACA AGT ATT GGG GAA AGC ATC Pro Thr Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Ser Ile Glu Ser Ile>				
2060	2070	2080	2090	2100
GAA GTC TCA TGC ACG GCA TCT GGG AAT CCC CCT CCA CAG ATC ATG TGG TTT AAA Glu Val Ser [Cys] Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys>				
2110	2120	2130	2140	2150
GAT AAT GAG ACC CTT GTA GAA GAC TCA GGC ATT GTA TTG AAG GAT GGG AAC CGG Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg>				
2170	2180	2190	2200	2210
AAC CTC ACT ATC CGC AGA GTG AGG AAG GAG GAC GAA GGC CTC TAC ACC TGC CAG Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr [Cys] Gln>				
2220	2230	2240	2250	2260
GCA TGC AGT GTT CTT GGC TGT GCA AAA GTG GAG GCA TTT TTC ATA ATA GAA GGT Ala [Cys] Ser Val Leu Gly [Cys] Ala Lys Val Glu Ala Phe Phe Ile Ile Glu Gly>				

FIG. 7G

FIG. 7H

2600	2610	2620	2630	2640
GTA GCA GTC AAA ATG TTG AAA GAA GGA ACA CAC AGT GAG CAT CGA GCT CTC	*	*	*	*
Val Ala Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg Ala Leu>				
2650	2660	2670	2680	2690
ATG TCT GAA CTC AAG ATC CTC ATT CAT ATT GGT CAC CAT CTC AAT GTG GTC AAC	*	*	*	*
Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu Asn Val Val Asn>				
2710	2720	2730	2740	2750
CTT CTA GGT GCC TGT ACC AAG CCA GGA GGG CCA CTC ATG GTG ATT GTG GAA TTC	*	*	*	*
Leu Leu Gly Ala Cys Thr Lys Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe>				
2760	2770	2780	2790	2800
TGC AAA TTT GGA AAC CTG TCC ACT TAC CTG AGG AGC AAG AGA AAT GAA TTT GTC	*	*	*	*
Cys Lys Phe Gly Asn Leu Ser Thr Tyr Leu Arg Ser Lys Arg Asn Glu Phe Val>				
2810	2820	2830	2840	2850
CCC TAC AAG ACC AAA GGG GCA CGA TTC CGT CAA GGG AAA GAC TAC GTT GGA GCA	*	*	*	*
Pro Tyr Lys Thr Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr Val Gly Ala>				
2870	2880	2890	2900	2910
ATC CCT GTG GAT CTG AAA CGG CGC TTG GAC AGC ATC ACC AGT AGC CAG AGC TCA	*	*	*	*
Ile Pro Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser>				

FIG. 7I

FIG. 7J

3250	3260	3270	3280	3290
GTG TAC ACA ATC CAG AGT GAC GTC TGG TCT TTT GGT GTT TTG CTG TGG GAA ATA				
val Tyr Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile>				
3300	3310	3320	3330	3340
TTT TCC TTA GGT GCT TCT CCA TAT CCT GGG GTA AAG ATT GAT GAA GAA TTT TGT				
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu Phe Cys>				
3350	3360	3370	3380	3390
AGG CGA TTG AAA GAA GGA ACT AGA ATG AGG GCC CCT GAT TAT ACT ACA CCA GAA				
Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr Thr Thr Pro Glu>				
3410	3420	3430	3440	3450
ATG TAC CAG ACC ATG CTG GAC TGC TGG CAC GGG GAG CCC AGT CAG AGA CCC ACG				
Met Tyr Gln Thr Met Leu Asp Cys Trp His Gly Glu Glu Pro Ser Gln Arg Pro Thr>				
3460	3470	3480	3490	3500
TTT TCA GAG TTG GTG GAA CAT TTG GGA AAT CTC TTG CAA GCT AAT GCT CAG CAG				
Phe Ser Glu leu Val Glu His Leu Gly Asn Leu Leu Gln Ala Asn Ala Gln Gln>				
3520	3530	3540	3550	3560
GAT GGC AAA GAC TAC ATT GTT CTT CCG ATA TCA GAG ACT TTG AGC ATG GAA GAG				
Asp Gly Lys Asp Tyr Ile Val Leu Pro Ile Ser Glu Thr Leu Ser Met Glu Glu>				

FIG. 7K

3570	3580	3590	3600	3610
GAT TCT GGA CTC TCT CTG CCT ACC TCA CCT GTT TCC TGT ATG GAG GAG GAA				*
Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu>				
3620	3630	3640	3650	3660
GTA TGT GAC CCC AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAG TAT CTG				*
Val Cys Asp Pro Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser Gln Tyr Leu>				
3680	3690	3700	3710	3720
CAG AAC AGT AAG CGA AAG AGC CGG CCT GTG AGT GTA AAA ACA TTT GAA GAT ATC				*
Gln Asn Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile>				
3730	3740	3750	3760	3770
CCG TTA GAA GAA CCA GAA GTA AAA GTA ATC CCA GAT GAC AAC CAG ACG GAC AGT				*
Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Asn Gln Thr Asp Ser>				
3790	3800	3810	3820	3830
GGT ATG GTT CTT GCC TCA GAA GAG CTG AAA ACT TTG GAA GAC AGA ACC AAA TTA				*
Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp Arg Thr Lys Leu>				
3840	3850	3860	3870	3880
TCT CCA TCT TTT GGT GGA ATG GTG CCC AGC AAA AGC AGG GAG TCT GTG GCA TCT				*
Ser Pro Ser Phe Gly Gly Met Val Pro Ser Lys Ser Arg Glu Ser Val Ala Ser>				

FIG. 7L

3890	*	3900	*	3910	*	3920	*	3930	*	3940	*
GAA GGC TCA AAC CAG ACA AGC GGC TAC CAG TCC GGA TAT CAC TCC GAT GAC ACA											
Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln Ser Gly Tyr His Ser Asp Thr>											
3950	*	3960	*	3970	*	3980	*	3990	*		
GAC ACC ACC GTG TAC TCC AGT GAG GAA GCA GAA CTT TTA AAG CTG ATA GAG ATT											
Asp Thr Thr Val Tyr Ser Ser Glu Glu Ala Glu Leu Leu Lys Leu Ile Glu Ile>											
4000	*	4010	*	4020	*	4030	*	4040	*	4050	*
GGA GTG CAA ACC GGT AGC ACA GCC CAG ATT CTC CAG CCT GAC ACG GGG ACC ACA											
Gly Val Gln Thr Gly Ser Thr Ala Gln Ile Leu Gln Pro Asp Thr Gly Thr Thr>											
4060	*	4070	*								
CTG AGC TCT CCT CCT GTT TAA											
Leu Ser Ser Pro Pro Val ***											

FIG. 7M

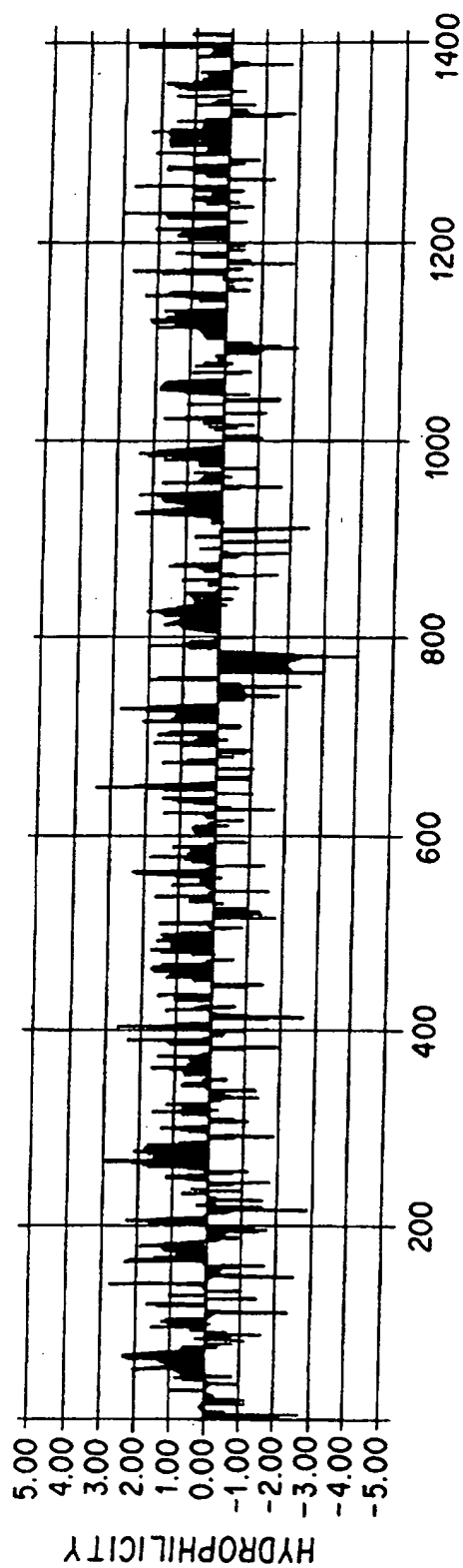


FIG. 8

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KDR 787 GTVKRANGGELKTGYLSIVMDPDELPDDEHCERLPYDASKWEFPDRRLNLGK
ckit 543 L**YLOKPMYEVQWKVVEEINGNNYVIDPTQ***H-*****N**SF**
CSF1 536 LLY*YKQPKYQVRWKIIESYEGNSYTFIDPTQ***NE-*****NN*QF**
PDGF 522 MLWQKKPRYEIRWKVIESVSSDGHXYIYVDPVQ***-ST*****QLV**R
      * * *
KDR 839 PLGRGAFGQIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILI
ckit 594 T**A***KVVAET*Y*LI*SDAAM*****PS*HLT*RE*****V*S
CSF1 587 T**A***KVV**T***LG*EDAVLK*****ST*HAD*KE*****MS
PDGF 573 T**S*****VV**T*H*LSHSQATMK*****ST*RSS*KQS*****MS
      * * *
KDR 891 HIGHHLNVVNLGACTKPGPLMVIVEFCKFGLNSTYLRSKRNEFVPYKTKG
ckit 646 YL*N*M*I*****I-***TL**T**Y*CY*D*LNF**R**DS*ICS*QED
CSF1 639 *L*Q*E*I*****H-***VL**T**Y*CY*D*LNF**R*AEAMLGPSLSP
PDGF 625 *L*P*****I*YI*T**Y*RY*D*VD**HRNKHT*LQRHSNK
      * * *
KDR 943 ARFRQKDYVGAIPVDLKRRLDSIT-SSQSSASSGFVEEKS-----SDV
ckit 697 HAEA-A-L*KNLLHSKESCS-DS*N-E-----YMDMKPGVS--YVVP--KA
CSF1 690 QDPE*GVDYKN*HLEK*YVRDSE**GVDYVEMRPPVSTSS-NDSE*EQ
PDGF 676 HCPPSAEL*SN*LP*GFSLPShLNLTGESDGGYMDMSKDESIDYVPMLOMKG
      * * *
KDR 987 EEEEEAPEDLYKDF-----LTLEHLICYSFQV
ckit 737 D-KRRSVRIGSYI-----ERDVTPAIMEDELA*D**D*LSF*Y**
CSF1 741 DLDKEDGRPL-----E*RD*LHF*S**
PDGF 728 DIKY*DIESPSYMAPYDNVPSAPERTYRATLINDSPV-*SYTD*VGF*Y**
      * * *
KDR 1013 AKGMEFLASRCKIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGD

```

FIG. 9A

F16.9B

IDENTIFICATION OF *kdp* mRNA

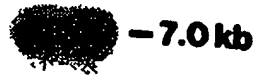


FIG. 10

**IDENTIFICATION OF *kdp* GENE
BY SOUTHERN ANALYSIS**



1 2 3 4

FIG. 11

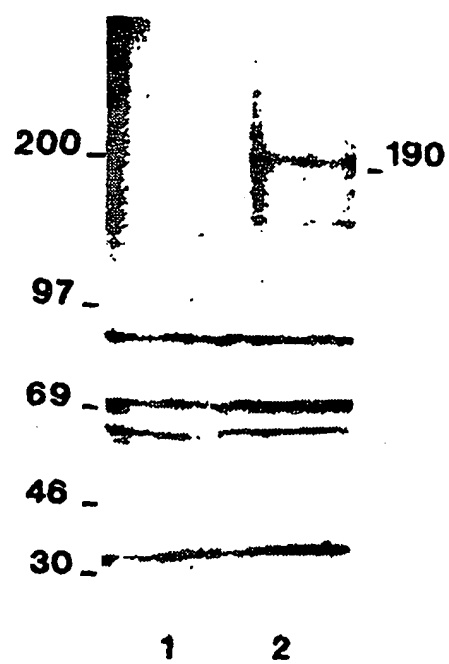


FIG. 12

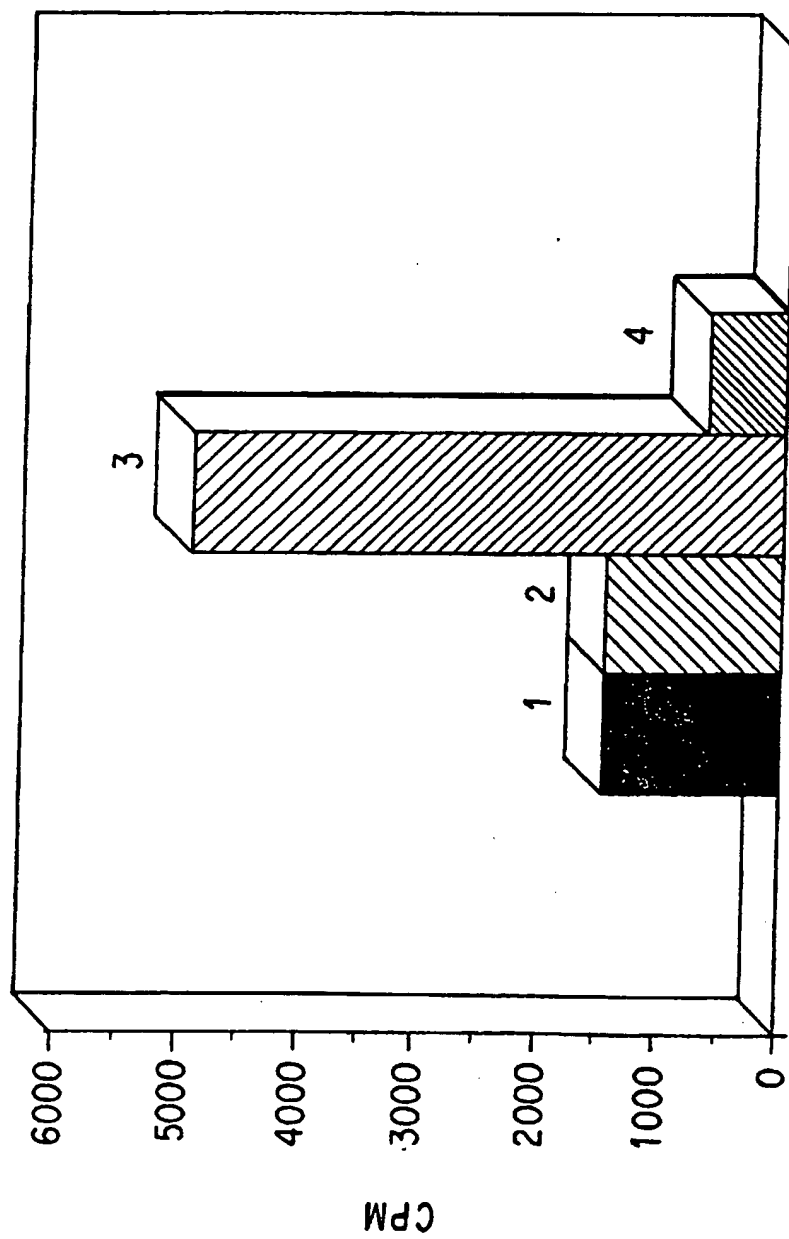


FIG. 13

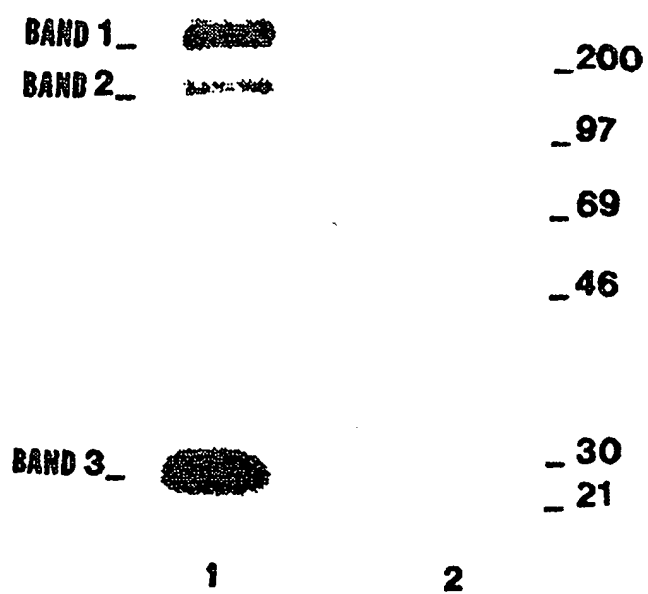


FIG. 14